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EDMUND B. WILSON—AN APPRECIATION

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WITH the passing of Edmund B. Wilson on March 3, 1939, at the age of eighty-three years, we biologists have lost him who long was our most beloved, revered and helpful guide and comrade in the great mutual adventure of exploring the world that is buried deep within ourselves and all living things. As leader, inspirer and teacher he was preëminent, as the many who were directly and profoundly influenced by him will attest. While many biologists were disputing whether chromosomes were permanent entities at all, he and Stevens provided the first definite and proved examples—those concerned with sex itself—of chromosomal inheritance. This work, taken in connection with the general theory of sex determination which Wilson based upon it, and with the correlative studies which he made on other chromosomes, marked the establishment of what we now call "cytogenetics," and therewith placed genetics in general on solid, visible ground.

Both in the prior preparation of the ground for cytogenetics and in the further elaboration of the theories erected on it, Wilson played a major role. His own observations, experiments and analyses supplied much of the factual and theoretical material required at the most critical points. His systematic work—carried on for over half a century—of evaluation, selection, coordination, his amazing recrystallization and ordering of the whole intricate mass of extant data and hypotheses deal-

ing either directly or indirectly with the all-inclusive problem of how the entire individual may lie implicit in the single cell, all this has fully stood the test of time and is an accepted and basic part of all modern theory in genetics, ontogenesis and related subjects.

Wilson's contact with science began when the establishment of the theory of evolution and even of the cell theory in its primitive form were still fresh in men's minds, and when these doctrines were actively serving for the reinvestigation or reassessment of all known biological facts. Mitosis and the chromosomes had not yet been discovered, nor even the fact that fertilization involves a union of two nuclei, one from each parent. For oil-immersion lenses and Abbé condensers, anilin dyes and section cutting had only just been invented. And experiment had hardly entered the fields of morphogenesis, cell study or evolution. As for the teaching of biology, it and other sciences were practically unknown in the schools and even in most of the smaller colleges (see Wilson, "Teaching and Research in the Natural Sciences," 1909). Thus Wilson's life spans practically the entire period of growth, not only of genetics, but of biology in general as we know it. Moreover, his own scientific activities largely illustrate this growth, for as improved methods of approach—either those of hand or brain—arose, Wilson time and again was to be found among the vanguard of those adopting them.

Although the childhood of "Eddy," as his parents called him, was spent in a small town—Geneva—on the prairies of Illinois, his home environment was conducive to the development of intellectual interests. For the parents of both his father, Federal Judge Isaac C. Wilson, and of his mother, Caroline Clarke Wilson, had brought with them from New England a tradition of education, general culture and music—without, however, any special background of science. And it is probably significant that many of his ancestors were sea-faring men of the old New England school, with their spirit of adventure, of independence and, above all perhaps, of skilful craftsman-

ship. Eddy's parents, as well as the Pattens—the child-
less aunt and uncle at whose house he spent much time—
were exceptionally sympathetic and understanding. With
his elder brother and his younger brother and sister he
spent a happy and wholesome childhood. He early
showed his scientific bent, particularly in the form of an
intense interest in animals, and his artistic bent, which
was directed especially towards music. His elders did
not discourage these interests. At six he was already
tending small animals he had caught; he kept them in the
room set aside for him at the Pattens, who drew the line
only at manure beetles. At sixteen, after a strenuous
year's experience teaching a country school, he was
already eager to devote his life to biology. The path
ahead was by no means free from material difficulties, but
by working summers as well as after hours at various
jobs, by winning fellowships and by the aid of an occa-
sional advance from his elder brother, Eddy managed to
gain the education that he sought.

After his year as a school teacher, Wilson entered upon
a period of increasingly good preparation, with particular
attention to biology, chemistry and other sciences, at vari-
ous colleges in turn. To most of these he was drawn by
the attractive accounts of them sent by his elder cousin,
Sam Clarke, who, likewise having biological leanings, had
preceded him there. The first college year he spent at
Antioch in Ohio, then a year at Chicago in preparation for
the Sheffield Scientific School of Yale, where he spent
three years. At Yale he studied under the naturalist Ver-
rill and, on the side, attended very stimulating lectures
on heredity and evolution given by the stock-breeder
Brewer. As a thesis for the Ph.B. degree he wrote two
descriptive papers on the Pycnogonids ("sea spiders").
During his last year there, in 1878–9, he saw for the first
time a picture of mitosis, in a work just published by Mark
on fertilization and cleavage in the snail's egg. This
proved to be the beginning of Wilson's permanent interest
in these matters.

After this he was delighted to secure a fellowship at the great graduate school of science that had not long before been established at Hopkins.   Here, chiefly under the influence of the magnetic and wide-visioned zoologist Brooks and of Huxley's former associate, the physiologist Martin, Wilson became accustomed to seeing beyond and beneath the circumscribed domain of traditional natural history.   From now on he began to fix, as his central objective, upon the problem of how the individual lies determined within the germ.   As he later (in "The Cell," 1896) expressed the matter, "that a cell can carry with it the sum total of the heritage of the species, that it can in the course of a few days or weeks give rise to a mollusk or a man is the greatest marvel of biological science."   In a sense, all the work which he subsequently did was in one way or another concerned with this question, in which both embryology and genetics lie epitomized.

Laying the Embryological Groundwork

In the years of Wilson's graduate work (1878–1881) and for some time thereafter the best available means of approach to this recondite problem appeared to lie in the accurate descriptive study of development.   This was a field in which much remained to be explored and which still seemed to hold promise of revealing many mysteries, both concerning the present relation of the individual to his germ and concerning the way in which, through past ages, germ and individual have come to be what they are. In this field, in his doctor's thesis giving the results of three summers' studies of the development of the colonial polyp *Renilla,* he did a distinguished piece of work, of highly critical and analytical character, using the technique, which had only just been invented, of section cutting.   Among other things, he showed that, despite the regular division of the nuclei, the cytoplasmic cleavage of the egg of this form is very variable, either segmenting the egg-surface definitely from the beginning or being
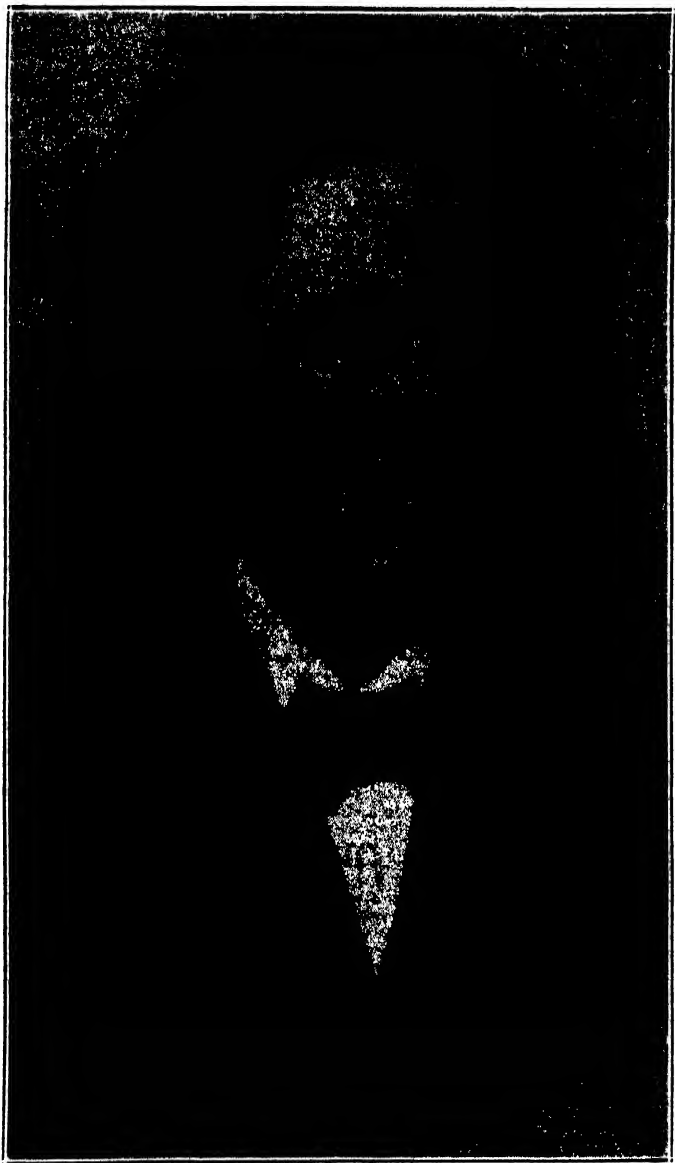
Wilson at eighteen, in his natural history days. At this time, 1875, he was an undergraduate at the Sheffield Scientific School of Yale.

more or less unexpressed until even the fourth division, when simultaneous demarkation of all the cells may occur; similarly variable is the spatial pattern of the cytoplasmic cleavage. In tracing the origin of muscle cells, spicules, mesogloea, etc., the absence of a separate mesoderm layer was confirmed. Interesting physiological, phylogenetic and ontogenetic conclusions were also drawn from the observations on the origin and nature of the different members of the colony. Shortly after the manuscript was completed, when Wilson was in Europe (1881–83), it won the commendation of Huxley, who introduced Wilson's presentation of it to the Royal Society.

In order to follow up Wilson's activities along this line in a consecutive way, we may for the moment pass over these two years in Europe, as well as the next two, spent mainly in teaching and writing, at Williams College (1883–84) and the Massachusetts Institute of Technology (1884–85). In 1885, when Bryn Mawr College was founded, Wilson secured an appointment as head of the department of biology there. This he held until 1891, when he was called to the department of zoology at Columbia by H. F. Osborn. At Bryn Mawr, thanks to the far-sighted educational policies of the dean, Miss Thomas, conditions for Wilson's research were again favorable. This enabled him now to follow up his work on *Renilla* by a similar study on the earthworm, *Lumbricus* (1887, 1889, 1890).

In his work on earthworm embryology Wilson traced the early stages in detail and succeeded in demonstrating that in this form and probably in annelids in general—contrary to some current reports—the mesoderm is formed by teloblast cells. This finding increased the significance of this feature of development for the determination of homologies and it helped greatly—especially in conjunction with Wilson's next embryological work, on *Nereis*—in indicating that most animals above the simple two-layered ones (Coelenterates) can be divided into two great stems. In one of these, the "teloblast series" (in-

cluding nemertines, flatworms, annelids, arthropods and mollusks), the members having the type of embryology more primitive for this group are characterized by a spiral, "mosaic" cleavage, in which specific cells are early set aside for teloblast cells, from the chainwise division of which the mesoderm is derived, while in the other, or "enteroblast series" (including *Sagitta,* Molluscoids, Echinoderms and Chordates) there is, primitively, a radial cleavage and the mesoderm is derived from pouches of the archenteron.

Wilson's modesty, and his caution in treading the already notoriously boggy ground of phylogenetic questions, prevented his laying emphasis upon the fundamental bifurcation of higher organisms thus indicated, although he called attention to it in the graduate course in zoology which he later gave at Columbia. But the fashion of regarding phylogenetic considerations as unimportant—which was beginning even in the early days of his embryological work, in reaction to the overconfidence shown by the first students of phylogeny—obscures what is probably a very important fact of animal relationships, the significance of which may be better appreciated in the future, with the progress of comparative physiology and biochemistry.

Wilson found the marine annelid *Nereis,* by virtue of the great precision and regularity of its pattern of cell divisions, far better adapted than the earthworm for tracing out the development of all parts in the minutest detail, cell by cell, from the fertilized egg onwards. His classical account of the development of this worm, published in 1890 and 1892, marks him as one of the most outstanding pioneers of "cell-lineage," as this new discipline was called. This study not only cleared up for the first time, in terms of the individual cells concerned, the origin of all the embryological structures in annelids having determinate cleavage. By a wider comparison of these results with those found by others in other types of teloblastic animals, such as flatworms and mollusks, Wilson showed

that while on the one hand there are far-reaching similarities in the whole peculiar cleavage-pattern and in the fate of the different cells of the cleavage stages shown by the widely different forms—understandable only as expressions of very ancient homologies—nevertheless there are also very striking differences between the larger groups, in that some of the parts which are obviously homologous in their later embryos are derived from non-corresponding cells of the early stages, even where these early stages themselves appear alike. In the phylogenetic history, then, one cell must at some time have become substituted for another as the primordium of a given tissue or organ. Thus the cell-lineages, though very valuable guides to homology and phylogeny, could no longer be regarded as absolute criteria, any more than the later stages. As Wilson subsequently emphasized (1894, 1895, 1898), the early stages must be capable of modification like the later, and so, while their remarkable parallelism in such widely differing forms must be ascribed to their long-continued retention of a common ancestral pattern of development (though not to the existence of any common ancestor the adult stage of which was like these embryos), this persistence of the ancient pattern must itself have been conditioned by the continuance of similar conditions of living, to which it was useful for these divers embryos to remain adapted.

In these conclusions Wilson had gradually branched out on to far deeper questions than those of purely descriptive embryology or even phylogeny. His activity in these more analytical fields, in which he was presently to resort to newer, more refined methods of attack, both observational and experimental, had been greatly stimulated by his most exhilarating stay abroad from 1881 to 1883—at Cambridge, Leipzig and especially at Dohrn's marine biological station at Naples. The 26-year-old Wilson had avidly absorbed the newer methods and ideas concerning the cell theory, the evolution theory and biology in general at the centers where they were being most

actively put forward.   He had at the same time greatly enriched his life by the international friendships which he formed, by the exotic associations born of his travel experiences and by the rapturous occasions on which he lost and found himself in the transports of music.   Such was the attraction to him of the mode of life thus opened up that he twice later, at intervals of approximately ten years, spent the better part of a year in Europe—in 1891–92 at Munich and Naples, and in 1903 again at Naples. From each visit he returned with greater intellectual stature and, conversely, his own influence on the Europeans increased markedly with each visit.   His example, moreover, did much to encourage the flow to and from Europe of other young biologists from America, which was then a comparatively backward country in science.   It can not be doubted that in the humbleness and in the internationalism of attitude exemplified in Wilson's European visits, in the willingness to learn from scientists in other countries, and in the increased opportunity thus afforded for integration of the best to be found everywhere, the United States owes much of its rise to a place second to none in the field of experimental and theoretical biology.   On the other hand, the more intense nationalism prevalent among the scientists of some European countries may in time have acted as a partial brake upon the progress of biology there.

INTEGRATIONAL WORK IN GENERAL BIOLOGY

In the two years between Wilson's first stay in Europe and his appointment at Bryn Mawr, having reduced facilities for research, he spent much of his time writing a "General Biology" (1885; second edition, 1895) in cooperation with his colleague, W. T. Sedgwick.   This was a work designed to introduce the study of life—both in its animal and plant manifestations—from a more modern and analytical point of view than that hitherto presented to beginning students.   The book started with a treatment of the nature of living matter which gave grounds

for inferring that it is composed of the same kinds of atoms as lifeless matter, working according to the same fundamental physico-chemical laws, but giving peculiar results because of its special, complex mode of organization. To quote from the 1885 edition: "For purposes of biological study life must be regarded as a property of a certain kind of compounded matter. But we are forced to regard the properties of compounds as the resultants of the properties of their constituent elements. . . . Reflections of this sort show how ignorant we are of the real properties of the elements and how important a further study of them is [an inference brilliantly vindicated by modern physical findings]. . . . The phenomena of consciousness . . . are not known to. occur apart from a living material basis with which they appear to be in some way closely connected" (pp. 5–6). "At every moment of its existence the organism is acted on by its environment; . . . at every moment it reacts upon the environment, maintaining with it a constantly shifting state of equilibrium" (p. 96).

Protoplasm, the cell and cell aggregates (tissues) were then presented on the above basis, that is, neither as static morphological patterns to be memorized nor as pure dynamics floating in a void, but as complexly organized working systems, their morphology and physiology being always considered in the closest relationship with one another. The same procedure was next followed in the detailed study of an example of a multicellular plant, the fern, and of an animal, the earthworm, in such wise that the student might always bear in mind the significance of each feature with reference to the part it played in connection with the other components in the workings of the whole individual and in the maintenance of the species. Everywhere it was shown—but not in the mystical spirit in which this point is sometimes made—that the parts of the organism "are mutually interdependent and . . . the organism as a whole is greater than its parts." Organisms of an intermediate degree of multicellular complexity

had been chosen in order, on the one hand, to avoid confusing the beginning student by excessive complications while, on the other hand, retaining the advantages of early consideration of organs and systems sufficiently specialized to bring the various functions into high relief. The method of presentation adopted made it easy for the student, on comparison of the earthworm and the fern with each other, to see that, far apart though they seemed superficially, practically all their obvious differences were accessory to their one most fundamental difference, that, in their mode of nutrition. For a student taking more biology the ground had now been prepared for a comparative view of different forms and for a consideration of the factors that had led them to become different.

The points of view in this book, although not actually novel at the time, need even to-day more emphasis and a wider dissemination than they commonly receive—witness the recurrent recrudescence of vitalistic and other mystical or crude ideas regarding living things. This was, however, much more true in 1885, when biology had only so recently begun to draw away from the animistic preconceptions of mankind in general, and when within its own domains its morphological and physiological sides were still on the whole so blind to one another. Fortunately the book had a wide influence. The 1900 edition can still be used with much profit in an introductory course.

EXPERIMENTAL ANALYSIS OF MORPHOGENESIS

As may be gathered from the above evidence of Wilson's interest both in physiology and in comparative morphology, he was one of the few biologists of that time to combine an appreciation of the importance of the experimental and of the comparative observational methods. As he pointed out, the two methods are after all not essentially different, inasmuch as the comparative one makes use of the "experiments carried on by nature," as he termed them in his lecture "Aims and Methods of Study

in Natural History'' (1900). ''The experiments performed in our laboratories but supplement those that have taken place and are always taking place in nature, and their results must be wrought into the same fabric'' (*ibid.*). Both means of approach are, he insisted, necessary and complementary, in view of the complicated organization of present-day organisms, representing as they do the accretion, by natural selection, of so many evolutionary steps. And, as he said later (1914) : ''What is the living organism, and how has it come to be? We often find it convenient to lay emphasis on one or the other of these questions . . . [but] fundamentally they are inseparable. The existing animal bears the indelible impress of its past; the extinct animal can be comprehended only in the light of the present.'' Being known to present-day geneticists mainly for his comparative studies on chromosomes, it is not so generally realized by them, however, that in the last pre-Mendelian decade (1890–1900) Wilson had already become one of the foremost in showing the value of experiment in attacking problems concerning the mode of organization of the germ cells. This was in accordance with his belief that ''the introduction of experimental methods into morphology is the most momentous step in biological method that has taken place since the introduction of such methods into physiology by Harvey and Haller'' (1900, *ibid.*).

The rich possibilities awaiting the use of experiments in this field had hardly been realized until Roux in 1888 started the experimentalist bark on its way. He showed that when blastomeres of the frog's egg are killed the surviving ones (for a time at least) develop ''mosaically,'' *i.e.*, into part of an embryo, as they would have done if in their normal setting. In 1891 Driesch countered by showing that isolated blastomeres of the sea urchin are ''totipotent,'' developing eventually into whole embryos. During Wilson's second stay at Naples (in 1891–92, just before taking up his new position at Columbia) he was plunged into the midst of this startling new line of work

on the cell theory. Joining at once in the experimental attack, he secured results on *Amphioxus* (1892–93) that indicated an even greater freedom from the limitations of mosaic development than seen by Driesch in the sea urchin.

But Wilson reacted differently from Roux and Weismann, on the one hand, who proceeded to "explain" their findings by setting up an elaborate scheme of segregation of hereditary materials, and from Driesch, on the other hand, who finally drifted to the mystical assumption of an "entelechy" within the cell. He had before him the lessons not only of these experiments but also of the findings which he had so recently made in his comparative studies on the development of *Nereis* and other teloblastic animals and which indicated that, although these cleavages were in a sense very definitely determined, they might at the same time, in a more ultimate sense, be plastic. Keeping his balance in the face of these cross-currents, he arrived at the more modest and yet truer conclusion (1893, 1899) that the manner of development of a given part is "a manifestation of the general formative energy acting at a particular point under given conditions, . . . the formative processes in special parts [being] definitely correlated with the organization of the entire mass." He did not suppose that such words explained the nature of the "field" or of the "organizer materials," as they are now termed, but this should not obscure the fact that there is a definite and important meaning here. That is, that the nature of a given part is determined only through a process of interaction between that and other parts of the whole. Wilson and O. Hertwig independently came to much the same conclusion on this matter (except that Wilson came to regard the interaction as to a considerable degree potentially independent of the state of division of the whole into cells) and for a time Driesch too accepted this view. It is easy now to overlook this meaning, because of the lack of knowledge of the nature of the interactions in question, and of the invisible or-

ganization resulting from them. The need for such knowledge was, however, realized by Wilson, more perhaps than by the others of that time.

One of the directions of attack on these problems lay in experiments in which the nuclei were shifted or interchanged in their positions. In some early experimental work on sea urchins Driesch (1892) had shown that when the arrangement of nuclei in cleavage stages is changed by pressure the resulting embryos nevertheless give rise to normal larvae. This was, however, not surprising in a form in which the blastomeres so long remain relatively undifferentiated. Wilson first verified this finding and then (1896) tried the same experiment on *Nereis,* where differentiation of blastomeres.had been so early evident. He found that even here normal larvae were produced, despite the fact that the nuclei had been so redistributed that many of them were contained in regions—and even in germ layers—other than those in which they normally would have lain. This greatly strengthened the conclusion concurred in by Wilson, Hertwig and Driesch that differentiation does not result from a segregation of nuclear material.

The ultimate equivalence of nuclei thus indicated remained, however, in striking contrast to the obvious segregation of developmental materials—now inferred to be cytoplasmic—which had been found in some of the experiments on separation of blastomeres. Thus, even in *Amphioxus* Wilson had found that the isolated blastomeres were far from uniform in showing immediate "total" development. And extreme mosaic types had now been found—for example, tunicates and the gasteropod *Ilyanassa,* both worked on by Crampton when a student of Wilson's—in which the development was much more regularly "partial" than in the amphibians that had been used by Roux to illustrate this principle. In what then lay the difference between these two types of development, termed by Conklin in 1898 the "indeterminate" and the "determinate" types? Could one say

that in the first there is an equivalence of blastomeres, together with "epigenetic" development, while in the second type development follows His's (1878) principle of "organbildende Keimbezirke" or Lankester's version, "precocious segregation"? Wilson considered it very unlikely that there should be a fundamental difference in the principles governing the development of the two seemingly so different types. He succeeded very well in reconciling the apparent discrepancies by referring them to variations in the time at which the underlying differentiations took place, in relation to the cleavage divisions.

Putting together all the facts available, Wilson concluded that development in all cases is fundamentally an "epigenetic" process, in that if the egg be traced back to an early enough stage of its formation—often, to an early stage within the ovary—its cytoplasm is found to be relatively undifferentiated regionally and even its polarity is dependent upon its relations with its neighbor cells. (Sometimes—as Wilson found to be the case in the echinoderm egg—this polarity is not the final polarity, which is dependent on the chance point of entrance of the spermatozoon.) In time, however, the egg must become transformed from this relatively amorphous condition into a cell or (depending on the timing of the process in relation to cleavage) group of cells in which the different "organ-forming materials" are to a greater or lesser degree sequestrated into different regions. By the time this happens, then, there is truth in His's contention that some kind of topographical organization exists which, though it may yet be invisible, helps to determine the later visible differences. Now if the broad features of this organization have already been laid down with considerable finality before cleavage commences— and if (as was later added) they are so arranged that parts of it will become separated by the early cleavage planes—the egg shows the type of development classed as "mosaic" or "determinate." Otherwise it will be classed as "indeterminate." In most cases, however,

some at least of the processes of differentiation in a sense never reach completion, even in the adult, but only approach an equilibrium condition that can still be upset at any time, as by cutting, with resultant regeneration, which after all represents only "a survival, in the adult, of a condition characteristic of the embryonic stages" (Wilson, 1898). In the embryo, the process of cell division often helps to demark the invisibly or visibly differentiated regions, yet the cells as separate units play only a secondary role in differentiation. As for the nuclei, those of the different cells, in the early stages of development at least, are to be regarded as equivalent in potency to one another and to the nucleus of the fertilized egg, even though each nucleus, within itself, must be highly organized and though the processes of differentiation themselves are ultimately referable to the activities of these nuclear materials.

It may here be pointed out that although Wilson's experiments along the above lines were mostly conducted upon eggs and embryos, they were not always confined to these stages. For example, Wilson in 1902 studied the mode of interaction of the parts by regeneration experiments on the adult stage of the polyp, *Renilla,* whose development he knew so well. He showed that in this material (as was by that time known for planarians) a direct transformation of given parts into others takes place, which is in this case dependent to some degree upon mechanical factors. In other experiments, transformation of germ layers (in the sense of parts normally formed by one germ layer now being formed by another) was noted by Wilson. All this emphasized the importance of the mutual interactions in determination, together with equivalence of nuclei.

The very fact that the above set of principles are today so taken for granted by biologists makes it the more difficult for them to put themselves back to the time when the road to their attainment was so beset with obstacles. Moreover, as different points had been originated by dif-

ferent investigators, many of them contending with one another and advocating elaborate schemes of their own, it was a task requiring especial objectivity, judgment and insight so to cull out, fit together and integrate the elements of value contained in the various erring, warring doctrines as to arrive at that synthetic point of view of development which we now have.

In 1902 at the South Harpswell Laboratory, Maine, and in 1903 during Wilson's third stay at Naples, he carried out an extended and striking series of experiments on the development of types with spiral cleavage: the Nemertean worm *Cerebratulus* (in Maine) and the mollusks *Patella* and *Dentalium* (in Naples). This work led mainly to a strengthening and extension of his previous conclusions. If cut in any direction shortly after maturation, before fertilization, the Nemertean egg proved to be equipotential in regard to the cytoplasmic determiners for all the distinctive features seen during the cleavage and larval (pilidium) stages. But if cut after fertilization it had become mosaic (*i.e.*, "organized" or "prelocalized"), as shown by the partial blastulae (nevertheless capable of considerable later regeneration) formed from such fragments as well as from isolated blastomeres. The development of pieces cut still later—*i.e.*, in the blastula stage —showed that by this time still further "prelocalization" had set in. Yatsu, carrying this work further, under Wilson's guidance, showed the gradualness of onset of these localizing processes in the *Cerebratulus* egg, their orientation along certain planes and the similarity in time and manner of prelocalization of the factors affecting later stages to those affecting the stages studied by Wilson.

In *Dentalium* and *Patella*, unlike *Cerebratulus*, Wilson found that the localization was already well advanced considerably before fertilization. A certain region in the lower half of the unfertilized egg had already come to contain materials necessary for the formation of a whole series of important parts of the larva. When this

region is cut away either in the unsegmented egg, when it is still unrecognizable, or in cleavage stages, when it periodically appears as a large "polar lobe," these parts do not develop at all. An exception to this rule is found in the apical organ. This is not formed in the region of the embryo corresponding to that part of the blastomere stage where the polar lobe was located, yet its formation is dependent upon the presence of the latter through the time of the two-cell stage; if, however, the polar lobe is not removed until after this stage the apical organ will be able to develop. The localization of the polar lobe material in the egg stages is only the earliest example of such processes here. As development progresses one premitotic localization follows another in the most elaborate fashion so that in fact each cell up to the 64-cell stage continues to form practically the same parts, if separated from the rest, as it would have done if left in its natural position in the whole. It thus becomes evident that localization of the materials necessary for the development of ever more circumscribed parts continues to occur. That is, "the divisions that would be considered as qualitative or quantitative from the point of view of descriptive cell-lineage, are really such as regards the inherent factors of differentiation. The descriptive and comparative study of cell-lineage represents something more, therefore, than a mere enumeration of successive cell divisions and their geometrical relations, and has the value of a direct examination of the normal morphogenic process'' (Wilson, 1904).

At the same time, the shapes, positions and sizes of the cells formed even by these extreme mosaic embryos were shown not to be entirely free from regulation by other parts, and this was, as expected, more evident in the earlier stages. For instance, an egg fragment or a $\frac{1}{2}$ blastomere containing the polar-lobe material could form virtually a whole larva, and even the size of the polar lobe and its derivatives formed by a fragment having originally a great excess of polar lobe material tended to be

normal in relation to the size of the other parts present. And the influence of the polar lobe on the formation of the apical organ seems to furnish another illustration of regulative action, as Wilson later (1929) pointed out.

A quarter century later, at the age of 72, Wilson resumed his experimental attack on these problems, studying the cleavage of the egg of the annelid *Chaetopterus* after its fragmentation by centrifuging. If the eggs were fragmented prior to a critical period that occurred shortly after their maturation, the majority of the fragments, on being fertilized, underwent normal cleavage (and some formed whole larvae) even though the visible materials had been abnormally distributed by the centrifuging and many fragments had received only the relatively clear "ground substance." Thus the conclusion could be drawn that it is in this clearer portion of the cytoplasm that the processes of pre-localization and differentiation normal to the embryo occur.

Once more reviewing the problem of localization versus regulation, it was shown in his last papers on the subject (1929, 1930) that the evidence had become stronger than ever both of pre-localization in the more markedly regulatory eggs and of regulation in those of more conspicuously mosaic type. It was also pointed out that, in fact, the two processes can not be considered as fundamentally distinct, both alike involving the production, more or less limited in time and in spatial distribution, of what were once termed "formative substances." The newer results on "organizers" likewise were shown to be of a piece with this general scheme; a probable illustration of this relationship was furnished by the manner of determination of the apical organ of *Dentalium,* above mentioned. The recognition of the superficiality of the distinctions here involved were, however, combined with a realization that this should only help in clearing the ground for the real work of experimental analysis of development, and especially of the peculiar "formative" processes that cause localizations of materials within definite regions of cells

or cell-aggregates to occur or not to occur, in accordance with whether or not certain other regions are present. Such work (as he had stated in 1914, then with reference to biological progress in general) would require that the investigator ''be thoroughly trained in the methods and results of chemistry and physics.''

In line with the above point of view and in opposition to Driesch and his sympathizers, Wilson on various occasions was led to reiterate (though usually in unprovocative language) his position that *''the scientific method is the mechanistic method''* (1903—italics his). It should be unnecessary to explain that this term was not used by him in the sense of crude mechanics, but in the same sense as usually employed by English-speaking scientific writers, namely, as the antithesis of the term ''vitalistic'' and so as practically equivalent to ''materialistic.'' Wilson was, however, too clear-sighted a scientist to be drawn into idle controversies over such terms.

That a ''mechanistic'' analysis (in the above sense) of fundamental cellular phenomena still had much to learn through a combination of exact observation and experiment on things of microscopic size, before these could receive their explanation entirely in the terms of the chemists and physicists, was, however, much clearer to Wilson than to those whose voices were beginning to be heard in favor of *''experiment alone''* (see Wilson's criticism of them in 1900), and some of whom refused to consider even an experiment scientific unless it made use of the technicalities of chemical or physical formulae. The short-sightedness and extreme oversimplification characterizing this position was in fact proved by nothing better than by Wilson's own work. In this connection we rightly think in the first place of the major work of his later years —that on chromosomes—but he was led to this gradually, from 1891 to 1901, as it became possible for efforts applied in this field to be more productive of results. In the meantime—and again in his later years—Wilson's main researches were along two lines correlative with this: (1)

the studies, above outlined, of the invisible organization of the cell in relation to the processes of early development, and (2) studies—also carried out mainly on eggs —of the properties of the visible achromatic constituents common to cells in general.

### Inquiries into the Material Basis of Mitosis

Examining the minutiae of cytoplasmic structure through their gradual transformations in the growth of the echinoderm and other eggs, Wilson found in 1899 that, although the cytoplasm finally becomes alveolar and thus comes to agree with Bütschli's conception of its universal conformation, this condition is attained by enlargement of extremely fine, scattered granules. A further substantiation was thereby provided of the view, for which some investigators were already contending, that neither an alveolar configuration nor a microscopically fibrillar or reticular one is fundamental or permanent.

In this and earlier papers (1895) on echinoderm egg material Wilson also paid particular attention to the achromatic structures connected with mitosis. It was demonstrated by him, partly in collaboration with A. P. Mathews, then his student, that Fol's "quadrille of centers" story of the origin of the centrosomes and asters at fertilization was wrong. Instead, it was shown, both asters of the first cleavage stage are derived from the sperm aster by division of the latter, and they in turn give rise, by their repeated division, to all the asters of later stages, as pictured in Boveri's scheme of the fertilization process. This material was, however, not well adapted for the observation of the central bodies within the asters. The evidence of various workers on other material seemed to show clearly the genetic continuity of these granules, yet the question remained in dispute. Long afterwards—in 1930 and 1931—Wilson returned to this question and—partly in collaboration with Huettner, who had been his student—gave clear-cut evidence of the persistence and reduplication of the central bodies

throughout the normal embryonic development of *Drosophila* and other forms.

As for the nature of the astral rays and spindle fibers, Wilson inferred from his observations of the 90's that they exist as actual fibers. Later, however (1901 *et seq.*), on the basis of newer observations and experiments showing the rapidity of the aster's movement and of its disappearance and reappearance with anesthetization (see below), he came to regard the rays at least as coagulation products and to favor a view of their representatives in the living cell as consisting of lines of flow or attempted flow, or at any rate of orientation, somewhat as had been proposed by Bütschli. Even in the earlier papers, however, he had not adopted the oversimplified fibrillar scheme of the mechanism of mitosis that had been advocated by Van Beneden and Heidenhain. For he argued that a mere fibrillar contractility of the spindle fibers could hardly cause the movement of the chromosomes to continue all the way to the poles, as he had observed it to do in the echinoderm eggs. He was therefore disposed to regard the movement as caused not by ordinary contraction of fibrils but by traction of some other kind.

In the late 80's and the 90's a series of novel observations and experiments on imperfectly fertilized and unfertilized eggs—by the Hertwigs, Ziegler, Mead, Morgan and others—had shown that eggs of species in which fertilization normally occurs are on occasion able to undergo maturation and/or some degree of cleavage without a sperm nucleus in the dividing cell and even without a spermatozoon at all. This presently culminated in Loeb's startling discovery of "artificial parthenogenesis," *i.e.*, that normal development can be artificially induced without a spermatozoon. Immediately thereafter, Wilson took up Loeb's method and, making the first accurate cytological study of the phenomena of artificial parthenogenesis, was able (1901) to throw new light on the nature of the mitotic structures and on the mechanism of mitosis. It had been thought that the centrosomes of
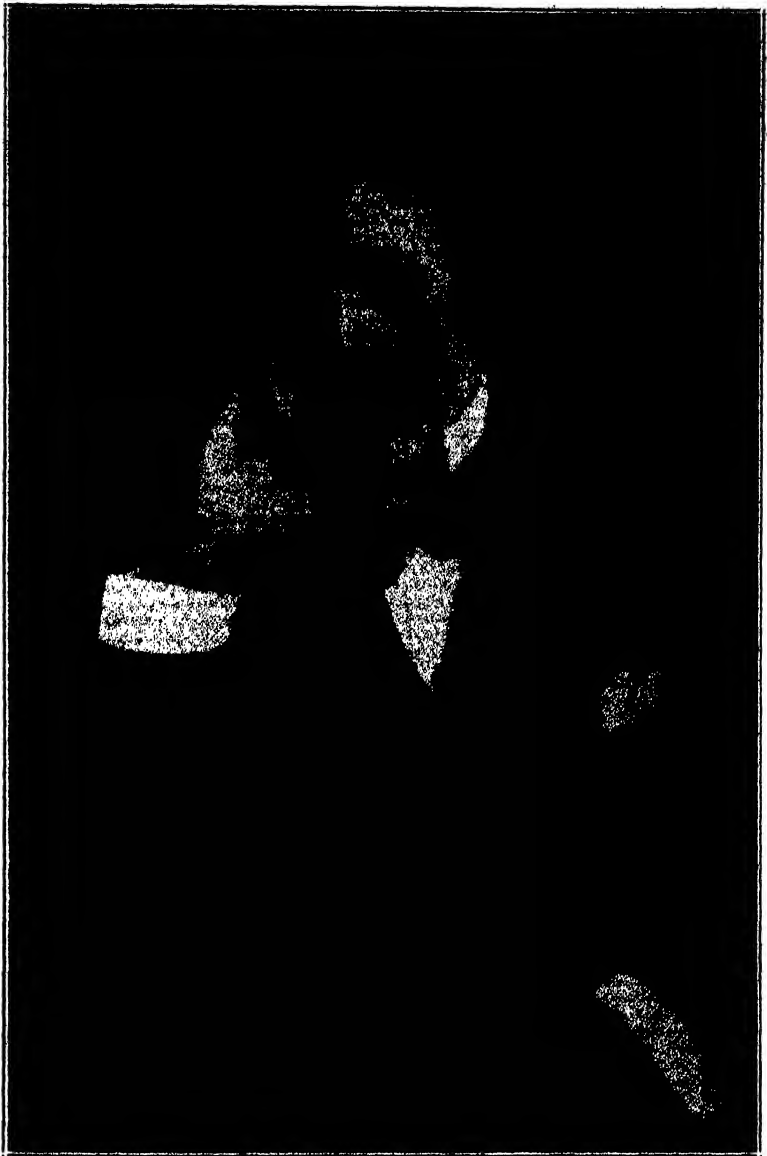
Wilson at thirty-three, in the heyday of his exploration of cell lineage. This was in 1890, his fifth year at Bryn Mawr, and his last before accepting the call to Columbia.

the cleavage stages were in this case formed from the achromatic portion of the egg nucleus, but Wilson (using the sea urchin *Toxopneustes*) showed that they arise in the cytoplasm (*de novo*), and he produced them even in egg fragments lacking a nucleus. Those that later became connected with a nucleus could act as normal centrosomes in cleavage, but artificial asters lying in "cells" lacking a nucleus (like the asters derived from sperm but separated from their nuclei in Boveri's earlier cases of "partial fertilization") also went through the motions of mitosis periodically. Without a nucleus, however, they seldom acted powerfully enough to effect complete cleavage of the cytoplasm.

When, as sometimes happens, centrosomes connected with a nucleus failed to divide, monasters were formed. Such figures had occasionally been seen in earlier work on fertilization, and it had been noted that they underwent some sort of pseudo-mitosis, but it was now shown by Wilson that in such cases not only do the chromosomes periodically condense but they undergo regular division with subsequent separation of the chromatids along the lines of the spindle fibers as in the early anaphase of regular mitosis. Thus the single reconstructed nucleus comes to have an ever doubling chromosome number.

It was evident from the above observations, taken together, that centrosome doubling and regular chromosome doubling do not necessarily depend, either one, upon the other. That there is, however, an underlying cytoplasmic rhythm in mitosis, to which the rhythm of both the centrosomes and the chromosomes may be secondary, was shown by Wilson in his work of a few years later on *Dentalium*, where he observed rhythmic changes in fragments of fertilized eggs lacking both centrosomes and nucleus, occurring synchronously with the mitoses in the complementary nucleated fragments.

Additional light on mitosis was shed by other experiments of Wilson's in the same year. In these he studied the effects of obliterating the astral figures in the fer-

tilized eggs by etherization—a phenomenon that had been discovered and to some event studied by the Hertwigs in the case of other anesthetics—and of obliterating the cleavage furrows by shaking—a method discovered by him. Both the movement of the pronuclei towards one another from a distance and the cleavage of the cytoplasm proved to be processes dependent upon the presence of well-formed asters, while separation of the two daughter chromosome-groups from each other was more or less complete according to the degree of development of the spindle. With moderate etherization or with shaking, division of the nuclei could occur without that of the cytoplasm. In cases of the latter sort, after recovery from the ether or from the shaking, not only might cleavage occur at the next mitosis along the planes cutting across the existing karyokinetic spindles, but also along the planes where all the previous cleavages (even remotely past ones) should have been—provided the asters still lay near enough to these planes. In the discussion, Wilson used the above and other results as further evidence for the exertion of a tractive force by the centers, similar in principle to that in Bütschli's hypothesis.

Preparing Cytology for Mendelism

Although Wilson had not yet, before 1900, carried out any major investigations dealing primarily with the "chromatin" (using this word here to denote the substance of the chromosomes, in general), he was not only very much aware of its importance, but had played an active part—among English-speaking scientists by far the most active part—in bringing this realization home to others. His own researches had had the effect of confronting him with cumulative evidence of the preponderant role of the nucleus in the ulterior processes of determination, and so had his careful studies of the literature. All this was, however, but a part of the organized view of cellular phenomena in their relation to the main problems of development, heredity, evolution and life

processes in general that he had now arrived at. Owing to his objectivity, his historical perspective and his faculty of extracting and integrating with each other the better elements of opposing ideas, this general conception was much in advance of anything that had previously been expressed in English and has in fact met the test of later work better than any other equally comprehensive synthesis of this field made at that time. Wilson's papers on individual problems could not, however, adequately convey his rounded conception, despite their exceptionally high value for historical orientation with reference to the given topics. It is therefore fortunate that, in his first decade at Columbia—1891 to 1900—Wilson spent considerable time in gathering together all this material and presenting it in the form of a monumental book, "The Cell in Development and Inheritance" (first edition, 1896; second, 1900). The basis for this book had been laid in a course of lectures given in 1892–93.

The book was dedicated to his dear friend Theodor Boveri, whom he had known at Munich and Naples and whose brilliant experiments and ideas on chromosomes had influenced Wilson more strongly than had the work of any other biologist of Wilson's own generation. "The primary intention of the work," as he stated in the introduction, was to contribute to the attempt (the keystone of which, according to Wilson, had been supplied by Weismann's arguments concerning the continuity of the germ plasm) "to bring the cell-theory and the evolution-theory into organic connection." This does not mean that it was intended to enter into any discussion of evolution itself, but to present the whole status of the cell-theory in such a way as to make it useful material for the further understanding of evolution and of those fundamental processes —such as heredity, variation and the determination of characters—on which it was becoming clear that evolution must be based.

How well Wilson succeeded in this purpose—considering the state of knowledge of that time—could not have

been nearly so evident then as to those reading the book
of 1896 or 1900 to-day, in the light of the now established
general theory of genetics. Not only would it be a good
thing for all beginners who expect to devote themselves
to genetics or related fields to read this book in an early
edition, but those already engaged in such lines who have
not read it for many years will find themselves well repaid
by a rereading of it now. Many of them will be amazed
at the foreshadowing of our modern conceptions therein
presented, and at how well the stage for genetics had
already been set. It will be forcibly brought home to
them what an accumulation of labor on the part of a
multitude of patient inquirers, and how many flashes of
insight—right and wrong—have been involved in the
establishment of our simple-seeming general theories,
how long a valid inference which is too good (too far ahead
of its time, as the saying is) must sometimes wait for
recognition, how very near a host of workers may come to
the understanding of the essentials of some important
phenomenon (*e.g.,* segregation, in pre-Mendelian days)
while still missing the key point (in this case, pairing of
likes) for the lack of some simple element (differences
between chromosomes of the same set) that apparently
might have been supplied by a very small step of reason-
ing, experiment or observation, how wrong and right at
the same time many a hypothesis may be (*e.g.,* Weis-
mann's idea of reduction or of the composition of chromo-
somes or His's idea of the organization of the egg), and
how valuable is the work of one like Wilson who, in bring-
ing the various facts and theories into due relation with
one another, is able to point out where solid ground lies
and to indicate in what direction further efforts might
usefully be exerted. Moreover, from a reading of this
book to-day, the reader is likely to emerge with an in-
vigorated confidence in the progress of science and an
increased respect, if not for men in particular, then for
man in general—a feeling that to-day may be sadly falter-
ing. And this respect will know no boundaries of nations.

"The Cell" gives a comprehensive treatment of the visible features of cell structure, mitosis, the formation of the germ cells, fertilization and the earlier stages of development, all in terms of the researches through which the facts were found, and with the aim constantly in view of shedding light on the nature of the basic life processes, on the mechanisms of development and of heredity, and so too, ultimately, on the process of evolution. The marshalling of the myriad facts leads Wilson, through a series of converging channels, to become increasingly emphatic, throughout the course of the book, in support of the conclusion—which, as he says, had been reached in 1884–85 by "Hertwig, Strasburger, Kölliker and Weismann independently and almost simultaneously"—"*that the nucleus contains the physical basis of inheritance; and that chromatin, its essential constituent, is the idioplasm postulated in Nägeli's theory*" (italics his). This is in fact rightly to be regarded as the central conclusion of the book (though in places Wilson still, with his characteristic caution, terms it "a working hypothesis")—much of the rest forming evidence in some way contributory to it or having a bearing on the manner in which the principle works out.

The chief lines of evidence for this conclusion then recognized by Wilson were the following (the same arguments being found in both the first hand and second editions) :

(1) The surpassing accuracy with which the chromosomes, by longitudinal division, *i.e., in all their parts,* are distributed at mitosis, in contrast with the cytoplasm, as pointed out by Roux in 1883 in arguing for the linear differentiation of the chromosome materials. This argument and certain supporting observations on the chromomeres and their division "point," said Wilson, "unmistakably to the conclusion that these granules are perhaps to be regarded as independent morphological elements of lower grade than the chromosomes." However, he agrees with Strasburger that the microscopically

visible bodies may not be the "ultimate dividing units." Of the latter he remarks: "Somewhere, however, the series [of smaller and smaller constituent parts of the chromosomes] must end in final chromatic units which cannot be further subdivided without the decomposition of chromatin into simpler chemical substances; and these units must be capable of assimilation, growth, and division without loss of their specific character."

(2) The indications, set forth by Rabl (1885) and Boveri (1887 *et seq.*), that the chromosomes, despite their seeming disappearance as such during the resting stages, retain some sort of individuality. The evidence of that time lay in the tendency of the chromosomes to reappear in their old positions after each resting stage in the series of the embryonic cell cycles, and in observations, made subsequently to this by Boveri, that at each reappearance they are of the same number and morphological types as they were to begin with, even when the original group of chromosomes had in one way or another been rendered an abnormal one. In 1895 Wilson had still been skeptical of the chromosomes' retaining their individuality, but by 1896, after Boveri's conclusions had received further support from findings of Häcker, Rückert and others of the separateness of the maternally and paternally derived chromosomes in the early stages of *Cyclops* and *Ascaris*, Wilson became a strong advocate of this doctrine. He was not yet convinced, however, that this individuality consisted in a retention of the actual identity of the chromosome material, and hence preferred to speak of the "genetic continuity" rather than the "individuality" of the chromosomes.

(3) "The whole process of maturation, in its broadest sense, renders the cytoplasm of the germ-cells as unlike, the nuclei as like, as possible. The latter undergo a series of complicated changes which result in a perfect equivalence between them at the time of their union, and, more remotely, a perfect equality of distribution to the embryonic cells. The cytoplasm, on the other hand, un-

dergoes a special differentiation in each to effect a secondary division of labor between the germ-cells. When this is correlated with the fact that the germ-cells, on the whole, have an equal effect on the specific character of the embryo, we are again forced to the conclusion that this effect must primarily be sought in the nucleus, and that the cytoplasm is in a sense only its agent.''

(4) The experiments of Nussbaum, Gruber, Verworn and others, in which cells of various kinds—protozoa, cells of plant hairs, etc.—were separated into nucleated and enucleated parts by cutting, plasmolysis and other means. These indicated that the presence of a nucleus was necessary for processes of constructive metabolism: growth, regeneration and apparently even assimilation. Moreover, observations on the position assumed by the nucleus in cells of different types and in different stages of their functioning indicated that the nuclei tend to lie in proximity to the regions of cells where active constructive processes are long continued.

As to the explanation of how the chromatin is given this superiority over the cytoplasm in development and heredity, Wilson realized that the problem was ultimately a chemical one. He discussed the chemistry of the chromatin and other constituents of the cell, as then known, in some detail, but made it evident that such work was not yet sufficiently advanced to disclose the mechanism involved. At that time, however, he leaned toward the hypothesis of Kossel, as carried further by Mathews (who, as Wilson's student, had in turn been influenced by him) ''that nuclein . . . may in a chemical sense be regarded as the formative center of the cell which is directly involved in the process by which food-matters are built up into the cell-substance'' and in ''the synthesis of complex organic matters.'' And this, as Wilson says, is ''in harmony with the hypothesis advanced twenty years ago by Claude Bernard (1878), who maintained that the cytoplasm is the seat of destructive metabolism, the nucleus the organ of constructive metabolism and organic syn-

thesis, and insisted that the role of the nucleus in nutrition gives the key to its significance as the organ of development, regeneration, and inheritance.'' Expanding upon this, Wilson says (in both editions alike):

In its physiological aspect, therefore, inheritance is the recurrence, in successive generations, of like forms of metabolism; and this is effected through the transmission from generation to generation of a specific substance or idioplasm which we have been reason to identify with chromatin. . . . If the nucleus be the formative centre of the cell, if nutritive substances be elaborated by or under the influence of the nucleus while they are built into the living fabric, then the specific character of the cytoplasm is determined by that of the nucleus, and the contradiction [that a specifically organized cytoplasm is necessary for the nuclear activity] vanishes. . . . The nucleus can not operate without a cytoplasmic field [sic!] in which its peculiar powers may come into play; but this field is created and moulded by itself.

The cellular biology of 1895 to 1900 was furthest behind that of to-day in its knowledge of meiosis. An important part of the clue to this great series of phenomena had, as Wilson recognized, been given by Weismann in his remarkable deduction of the existence of a reduction division, effecting a qualitative separation of hereditary (chromosomal) constituents. Unfortunately, Weismann had depicted this as involving either transverse division or separation of chromosomes. The observations seemed in part to tally with the idea of some sort of qualitative reduction, but the various accounts of the complicated conformations of meiosis were extraordinarily difficult to interpret and to reconcile with one another, since it was assumed by those who already believed in a binary conjugation of the chromosomes that the juxtaposition was endwise. For neither the conception of parasynapsis (Winiwarter, 1900) nor that of homologous chromosomes (see below) had yet been launched. The side-by-side conjugation plainly seen in some objects was thus mistaken for longitudinal division, and the resultant appearance of double longitudinal division at meiosis in these cases seemed to leave no room for reduction at all. On the other hand, Weismann's idea of transverse chromosomal division at reduction, which was widely interpreted

as separation between chromosomes that had been joined end to end, was supposed to have been proved in some cases, and as an explanation of reduction it fitted in well (without the necessity of postulating homologous chromosomes) with his over-elaborated idea of chromosome structure.   According to this, each of the numerous chromomeres or "ids," in every chromosome of a germ cell, contains a complete set of determiners for the individual.   On this conception the chromosomes must all be nearly equivalent and conjugation need not be precisely ordered.

Perhaps it was the diversion of attention caused by this idea that so long retarded the discovery of parasynapsis and of the simple fact that the different chromosomes of a given individual are often recognizably different from one another.   The latter observation, combined with the already extant doctrines of equivalence of male and female germ nuclei and persistence of chromosome individuality, must rapidly have led to the conception of homologous chromosomes and then to its corollaries, synapsis and segregation of the homologues.   The existence of pairs of homologous maternal and paternal chromosomes was in fact finally proposed by Montgomery, in 1901, apparently independently of Mendelism. Had this happened a year sooner, "Mendelism" would have been independently arrived at by cytologists, and the confusing cytology of meiosis might sooner have been disentangled.

But despite these pre-Mendelian difficulties, some of which, like the claim that there is a double longitudinal division in meiosis, greatly disturbed Wilson, he declared that:

The peculiarities of the maturation-mitoses are obviously correlated in some way with the numerical reduction, and the fact that they differ in so many ways from the characters of ordinary mitosis gives ground to hope that their exhaustive study will throw further light not only on the reduction problem itself but also on mitosis in general and on still wider problems relating to the individuality of the chromosomes and the morphological organization of the nucleus.

In accordance with this view concerning the direction of the trail ahead, Wilson himself, with some of his students—*e.g.*, Calkins and McGregor—were already actively at work upon the cytology of the maturation stages, in a variety of forms.

On the last page of both early editions, Wilson called attention to the complete lack of knowledge of the time concerning the mechanism of variation and to the fact that ''study of the cell has on the whole seemed to widen rather than to narrow the enormous gap that separates even the lowest forms of life from the inorganic world.'' He expressed his ''conviction that the magnitude of the problem of development, whether ontogenetic or phylogenetic, has been underestimated.'' Nevertheless, he concluded, ''the splendid achievements of cell-research in the past twenty years stand as the promise of its possibilities for the future, and we need set no limit to its advance. To Schleiden and Schwann the present standpoint of the cell-theory might well have seemed unattainable. We cannot foretell its future triumphs, nor can we doubt that the way has already been opened to a better understanding of inheritance and development.''

(*To be concluded in the March–April issue*)

ADAPTATIONS IN THE NASAL PASSAGES FOR SAND BURROWING IN THE SAURIAN GENUS *UMA*[1,2]

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MOSAUER (1932) in his study of adaptive convergence in the sand reptiles of the Sahara and of California gave considerable attention to certain of the sand adaptations in *Uma notata,* the Ocellated Sand Lizard. However, the nasal structure of this lizard has been given only a cursory examination. The writer's investigation is directed toward a more complete study of the olfactory apparatus both as to structure and functioning in an effort to explain successful subsurface respiration in this genus.

The lizards of the iguanid genus *Uma* are confined to deserts of the southwestern United States and Sonora, Mexico. They are characterized by elongate, pointed scales on the sides of the digits and an ocellated dorsal pattern.

These reptiles inhabit aeolian sand, characteristic of arid regions (see Fig. 1), and have developed the habit of submerging beneath the surface of this material for protection. Life in the sand has left its mark in numerous adaptations, one of which has given rise to the vernacular name "Fringe-foot."

These lizards spend much of their time beneath the ground. Since the animals are surrounded by minute, freely movable particles which are smaller in size than the external nares, a problem of respiration beneath such material is encountered. These facts led to the examination of the nasal apparatus. The existence of a device whereby small particles are filtered out of the inspired air was suspected.

[1] Condensation of a thesis submitted in partial fulfilment of the requirements for the degree of master of arts. Work carried on under the direction of Professor R. B. Cowles, of the Zoology Department, University of California, Los Angeles.

[2] Illustrations by the author.

The following account describes first the gross and microscopic structure of the nasal passages and adjacent features and secondly the function of these channels in one aspect of the ecology of these reptiles.

·Following the review of the genus *Uma* made by Heifetz (1941) *Uma notata notata* and *U. inornata* have been used most extensively in this study. No appreciable difference between these forms has been detected in
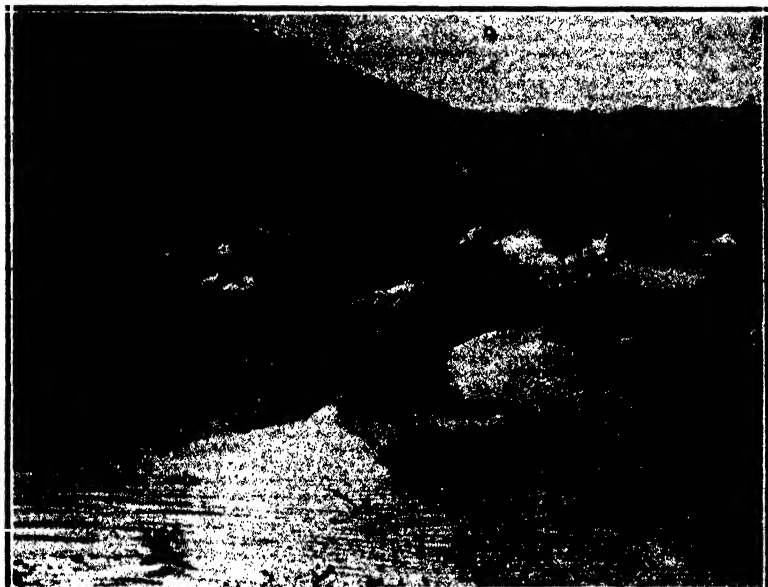

Photo by R. B. Cowles

Fig. 1.   Hummocks of wind-blown sand resulting from the deposition of particles impeded by scattered vegetation. *Uma inornata* frequents this deposit located a few miles northwest of Thousand Palms in the Coachella Valley.

the morphology and function of the nasal passages. *Uma scoparia* has been given a cursory examination. This species is similar to the other two and therefore the statements made here are equally applicable to all the known members of the genus.

ADAPTATIONS IN THE NASAL STRUCTURE

Uma has successfully coped with the problem of respiration beneath a medium which in its mechanical proper-

ties has a general resemblance to those of a fluid. The largest particles of sand are much smaller than the nostrils of the lizard and the smallest fragments approach in minuteness particles which can be called dust. Respiration is carried on underground for hours at a time without any apparent inconvenience to the reptile. The nasal mechanism and its functioning is such that sand is prevented from reaching the lungs. Thus these vital organs are adequately protected from foreign particles.

Gross Morphology

In *Uma* the nasal passages are tubular, forming U-shaped channels. The nostrils are situated dorsally and anteriorly on the snout. They are elliptical in shape with their long axes at an angle to the main axis of the head, the anterior edges diverging.

To facilitate description, the path of only one of these paired structures will be followed. Attention to the illustrations (Plates I and II) is essential for an understanding of this rather complicated breathing tube.

The external naris opens into a passage, the diameter of which is about the same as that of the nostril. This passage extends medio-ventrally, turning abruptly and continuing posteriorly just beneath the bones of the nasal region to a point near the eyeball. The passage doubles back on itself and proceeds anteriorly with a constant diameter approximating that of the dorsal limb of the tube, terminating in a depressed, oval chamber, the principal nasal cavity. On the antero-lateral portion of the floor of this chamber is located the naso-pharyngeal canal which extends at an angle, medio-ventrally into the mouth by way of the internal naris (Plate II, Fig. 2).

A conspicuous feature of the gross morphology of the nasal region is a large gland, the glandula nasalis lateralis, which rests upon the dorsal wall of the lower limb of the nasal tube adjacent to the dorsal limb (Plate I, Fig. 1). This gland opens by two ducts at the extreme posterior portion of the lower limb.

Microscopic Structure

Nasal Valve: The region of the vestibule adjacent to the external naris is highly vascularized. Blood and lymph flowing into the tissue of the floor of the vestibule proximal to the external nasal aperture cause a swelling which may almost completely block the opening. This valve-like floor possesses an abundance of smooth muscle fibers which underlie a stratified squamous epithelium. The region is free of glandular elements.

Epithelium of the Nasal Passages: Vestibule. The lining of the dorsal limb of the nasal passage is a stratified squamous epithelium which is uniform in thickness throughout. It is devoid of glands. This layer lines the cavity of the vestibule which doubles on itself posteriorly, extending to the anteriorly situated oval chamber, the principal nasal cavity (Plate I, Fig. 1). This cavity, along with a small segment of the vestibule, together comprise the ventral limb.

Principal Cavity: The oval chamber itself is lined throughout with a ciliated, pseudostratified epithelium composed of two types of cells, those which comprise Bowman's glands, the multicellular and unicellular mucus secreting structures and likewise the non-glandular, ciliated elements. The cilia uniformly cover the entire inner surface of the principal cavity (Plate II, Fig. 1). These cilia are extremely abundant and are short and thick, almost bristle-like in appearance.

Cilia extend posteriorly along the roof of the mouth. This ciliated epithelium is largely confined to grooves which extend posteriorly from the internal nares. These grooves converge medially to form a single channel which passes toward the throat (Plate I, Fig. 2, and Plate II, Fig. 1). No cilia are present anterior to the choanae.

Glandula nasalis lateralis: The tissue of the lateral nasal gland is comparable in structure to mucous gland tissue in mammals. The gland is composed of numerous tubules lined with cuboidal epithelium. These tubules lead to larger ducts which are lined with columnar epi-

thelium. These larger channels finally unite to form two master ducts which pour the secreted products into the nasal tube just above the principal nasal cavity (Plate II, Fig. 1). The tubules and ducts are held together in a matrix of loose, parenchyma tissue.

Walls of the Nasal Passages: The walls of the nasal passages of *Uma* are highly vascularized. Numerous vessels form a network surrounding the nasal tubes. Smooth muscle fibers, arranged for the most part with their long axes at right angles to the nasal epithelium, are in a position to dilate the nasal tubes by their contraction. These fibers are attached to the nasal epithelium on the one hand and to the adjoining connective tissue system, cartilage, fibrous connective tissue or bone as the case may be on the other. An influx of blood into the vessels about the tubes would cause a narrowing of the passages. The nature of the vestibular tissue is not unlike erectile tissue as found in certain mammals. A mechanism is thus present for the control of the diameter of the nasal tubes.[2]

Function of the Nasal Apparatus in Uma

Sand grains are irregular in shape and size and when dry are freely movable over one another. Under these conditions air spaces occur continuously throughout the fragments. There is thus an abundance of air in the interstices between the sand particles which compose the substratum of the habitat of *Uma*. The problem is to obtain this air during subsurface respiration without at the same time drawing particles of sand into the vital respiratory structures.

[2] Bruner (1907) describes a sinus vestibuli nasi which occurs in the region of the external naris in certain reptiles. This vascularization of the tissues of this region comprises a portion of a general system of cephalic venous sinuses present in reptiles. Briefly it may be stated that these sinuses can be dilated by contraction of a ring of striated muscle fibers about each of the internal jugular veins which causes a damming up of the blood in the head. The vascularized walls of the nasal passages in *Uma* constitute a portion of this system of cephalic veins. The same stimulus that initiates an influx of blood into the sinus vestibuli nasi resulting in an elevation of the floor of the nasal passage adjacent to the external naris (page 41), doubtless concomitantly causes a reduction in the diameter of the nasal tubes.

·When *Uma* lies buried near the surface and is respiring rather vigorously, funnel-shaped pits form in the sand over each nostril (see Fig. 2).   Examination of the nostrils, which appear occasionally at the bottom of the pits during expiration, reveals that the apertures have been narrowed considerably by elevation of the floor of the nasal tubes.   However, it has been observed that particles move freely back and forth through the crescentic
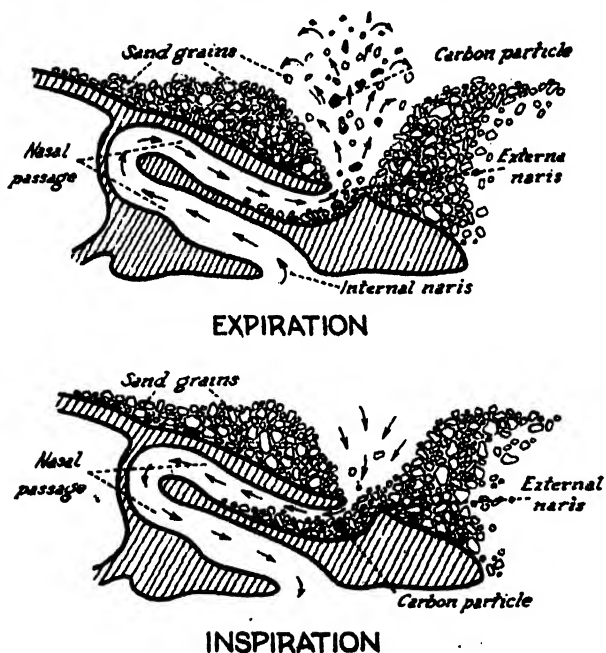

EXPIRATION

INSPIRATION

FIG. 2.   Semi-diagrammatic representation of a sagittal section through one of the nasal passages of *Uma* showing the movement of sand particles during respiration at a superficial level in the soil.

openings regardless of the valvular action.   The function of the valves minimizes but does not completely stop the entry of grains.

With each expiration, which is quite explosive in character, the sand is blown into a jet of particles which rise and then fall back about the periphery of the funnel as well as into the bottom of the pit.

Upon inspiration the particles begin to move inward but soon interfere with one another, massing together

with the result that only an occasional two, three, or a half dozen grains disappear from view. Nevertheless, even if only a few particles were sucked into the nasal apparatus at each inspiration, in the course of a few hours an appreciable accumulation of sand would occur.

With this possibility in mind the fate of an individual grain was determined by carefully removing sand from the region of the head of a specimen which had just buried itself. This was done so as to leave only a thin layer of material over the external nares. Pits due to respiration were immediately formed over each naris. Within each pit the sand oscillated up and down with respiration. A particle of carbon, about the size of a sand grain, was placed in the pit. This fragment could be easily distinguished as it oscillated in a vertical plane with the other particles. Occasionally it disappeared into the nasal aperture but it invariably reappeared. This oscillation of the carbon particle continued for 20 minutes (see Fig. 2).

A repetition of the above experiment was performed with similar observations. This time a carbon particle was placed in each pit formed by respiratory action. After a few minutes of examination, with the results confirming the previous experiment, the pits were carefully covered with sufficient sand so that oscillation of particles was prevented. After several minutes the sand was removed revealing the carbon fragments at the external nares in a position corresponding to that observed in the open pits. It was thought that the added weight of overlying sand might cause inhalation of those sand particles near the nasal aperture.

The carbon particle experiment indicates that little sand reaches the lower limb of the nasal tube. A number of factors are involved: (1) A purely physical phenomenon, separate from any adaptive features of the nasal structure, is of considerable importance in the explanation of the successful subsurface respiration of *Uma*. There is a tendency for the angular sand fragments to

interfere with each other as they are drawn toward the nasal aperture with the resultant formation of a grill of interlocking particles which tends to prevent the inspiration of other fragments. (2) Ordinarily when *Uma* lies buried beneath the sand, the nasal tube is at an angle to the horizontal (see Fig. 2). Sand grains which reach the vestibule must be drawn up an inclined plane in order to reach the lower portion of the channel. They must be moved against the pull of gravity. The vestibule is without glandular elements, hence it is essentially dry, nevertheless its walls frequently exhibit a few adherent particles. However, most of the particles drawn in with inspiration are loose and for the most part are freely blown out with expiration. (3) The grains which adhere to the walls of the nasal tube in proximity to the external naris and about the aperture serve to impede the inward progress of free grains. (4) The valve at the external naris functions to reduce the amount of sand gaining access to the passages but does not completely prevent its entrance. This capacity was demonstrated by holding a living lizard with the nostril on one side of the head directed upward. A small amount of sand was poured into the opening. The result was an elevation of the floor of the passage, reducing the nostril to a narrow, crescent-shaped slit (Plate II, Fig. 1). The arrangement of the smooth muscle fibers in the nasal valve of *Uma* is such that with contraction there is a depression of the epithelial surface in this region. The elevation of the floor of the nasal passage results from an influx of blood and lymph into the sinuses of the valvular tissue in a manner suggested previously in connection with swelling of the lining of the nasal tubes. Bruner (1907) believes that the cephalic damming up of the blood dilates certain sinuses in the orbital region and elsewhere, facilitating exuviation by stretching and ultimately tearing the skin. The peripheral stimulation of loose skin about the head apparently initiates the reflex. It is possible that the presence of sand grains in the nasal aperture and about

the head may set off a similar reaction leading to dilation of the sinuses about the passages with attendant constriction of the nasal channels. (5) The narrowing of the nasal tubes would further impede the inspiration of sand grains.

Respiration itself is of such a nature that it favors the removal of sand from the nasal passages. (6) Inspiration is gradual. This has been concluded from observation of thoracic movements and the oscillation of sand particles over the nostrils. With inspiration the ribs move slowly outward and the grains of sand in the nostrils are scarcely disturbed. A small amount of material may be drawn into the nasal passages but with expiration the ribs move inward, at first rather slowly, later terminated with a vigorous contraction which drives the air forcibly from the lungs. The sand grains in the distal portion of the nasal tube are moved outward and the particles over the nostrils are forced away from the nasal opening.

The shape of the passages is such as to accentuate the effect of forceful expiration. The shape of a nasal passage when straightened out from the U-shaped configuration is roughly that of a cone. The expanded end is that which opens into the buccal cavity and corresponds to the principal nasal cavity. Air forced through the larger end of such a tube increases in velocity toward the constricted portion of the cone. The high velocity in this region carries away sand particles. Air drawn in with inspiration through the external naris rapidly decreases in velocity in the direction of the expanded portion of the cone resulting in deposition of sand grains. Most deposition occurs in the vestibular portion of the nasal passage which in part is due to gradual inspiration.

The lizard makes use of the previously described configuration in eliminating residual particles in the dorsal limb of the nasal tube. Upon coming to the surface, sand may be blown from the nostrils and the valve is usually depressed. Tongue action may be involved in compres-

sion of the air in the principal cavity. The air leaves the external naris with explosive velocity carrying with it any loose material which may be present.

As was pointed out in the description of the carbon particle experiment, under deeper soil conditions, funnels do not form over the nostrils. In other respects respiration is similar. Most of the facts previously brought out in connection with breathing under shallow soil conditions hold as well for respiration at greater depths.

On a number of occasions at both superficial and deeper levels specimens have been found to possess caps of encrusted sand over the nostrils. Sand grains become adherent apparently from moisture from the nasal tube. External conditions of humidity and temperature are associated with the degree of adherence of particles. Spaces occur between the agglutinated grains and there seems to be no interference with respiration. Under these conditions the freely movable, dry particles are prevented from entering the nasal tube by this grill of adhering fragments.

Up to this point attention has been largely directed toward the structure and function of the vestibule of the nasal passage with respect to subsurface respiration.

Dissection of a number of specimens has revealed that the lower portion of the nasal tube tends to remain free of sand. In a few cases a small deposit has been found, but in general the particles fail to pass around the abrupt bend in the nasal passage. If particles, though few in number, were allowed to accumulate indefinitely a sizable deposit would result and obstruction to breathing would ensue. It is not logically conceivable that these fragments which have reached the mucus covered epithelium of the principal cavity are blown out through the external naris. These grains are enveloped in mucus and are carried in such a film toward the internal naris.

The fluid secreted by Bowman's glands, which are located in the walls of the principal cavity, is augmented by the secretion from the lateral nasal gland which opens by two ducts just above the nasal cavity. The cilia beat

toward the internal naris and the sheet of mucus moved by their action conveys the particles of sand into the mouth and thence into the throat to be swallowed.

Sand in the digestive tract is not uncommon. It has been found in the stomach and in the faecal masses. Ingestion of grains often occurs in feeding. It is assumed that no ill effect would result from the swallowing of sand-laden mucus.

The glottis of *Uma* lies in a depression at the base of the fleshy tongue. The floor of the orbit projects inward on either side of the roof of the mouth cavity. Between the orbital projections there occurs a narrow furrow. When the mouth is closed the glottis is situated in this trough. The glottis is valvular. Two dorso-lateral wings joined at their upper edges swing inward ventrally to close the trachea. The presence of this valvular mechanism for closing the opening of the trachea acts as a final barrier against inspiration of foreign material.

CONCLUSION

Comparative studies reveal that certain other genera of the Iguanidae possess a similar type of nasal apparatus. The genera *Uta*, *Sceloporus*, *Phrynosoma* and *Callisaurus* are almost identical in structure to the genus *Uma*. At the same time there are genera of the Iguanidae which diverge considerably from the Uma-type. Examination of many other saurian families revealed no cases of similarity in olfactory configuration to the U-shaped passages of the preceeding genera of the Iguanidae.

Lubosch (1934) mentions that the vestibule of the olfactory organ may be an adaptation to the problem of removal of dust from inspired air, associated with the terrestrial habit. The amphibians have a very poorly developed approach tube. If this is true an exaggerated vestibular element of the nasal structure might logically be expected under conditions where the 'dust removal problem is greatly intensified. Such conditions would occur in subsurface respiration beneath fine, loose parti-

cles. In the Uma-like members of the Iguanidae this trend seems to have occurred. The olfactory chamber is essentially a long tube, the principal nasal cavity appears as a relatively small dilation. The displacement of the nasal cavity to a position ventral to the vestibule may be associated with the elongation within a limited space of the approach tube.

Most of the forms which possess a *Uma*-like nasal structure often submerge beneath finely divided soil. It is suggested that this tendency may be in part correlated with an efficient sand trap which facilitates respiration beneath loose material. The acquisition of the sand extracting mechanism doubtless was of great importance in the adoption of the almost completely psammophilous habit by the iguanid genus *Uma*.

SUMMARY

Members of the iguanid genus *Uma* possess looped, U-shaped nasal passages which in structure and functioning prevent the inspiration of sand particles during subsurface respiration.

The following factors are involved in the exclusion and elimination of sand particles:

(1) The sand particles interfere with one another as they are drawn toward the external naris during inspiration. There is a tendency toward the formation of a grill of interlocking fragments which obstruct other loose grains.

(2) When *Uma* lies buried the vestibule is situated at an angle to the horizontal. The sand particles must be drawn up the approach tube against the pull of gravity before reaching the principal nasal cavity.

(3) Sand grains which adhere to the walls of the vestibule tend to impede the movement of free grains.

(4) Under conditions of subsurface respiration the valve at the external naris reduces the nasal aperture to a narrow crescentic slit, limiting the inspiration of particles.

(5) The postulated reduction in the diameter of the nasal tubes concomitant with the valvular action of the floor of the vestibule adjacent to the external naris, may tend to obstruct sand particles.

(6) Gradual inspiration coupled with explosive expiration aids in avoiding the accumulation of sand in the nasal tubes. The configuration of the olfactory channels accentuates the explosive effect of expiration.

(7) The small number of sand grains reaching the principal cavity are conveyed in a mucous film moved by ciliary action into the mouth, ultimately passing into the digestive tract.

The genus *Uma* is not unique with respect to nasal structure. A number of other genera of the Iguanidae were found to possess a nasal configuration closely similar to that occurring in this genus. *Callisaurus, Phrynosoma, Uta, Sceloporus* are such genera. A tendency toward soil submergence occurs among these groups as based on field and laboratory observation. Other non-burrowing[3] genera of the Iguanidae exhibited a nasal configuration quite different from the Uma-like types.

The occurrence of the looped nasal structure within the Iguanidae doubtless was of considerable importance in the development of the almost completely psammophilous habit of the genus *Uma*.

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[3] Burrowing is considered in the sense of submergence or ''soil swimming'' rather than in the sense of tunneling or the excavation of a cavity for retreat.

(EXPLANATION OF PLATES I AND II)

Natural size of specimen from which drawing was made measured 7 mm from posterior eye corner to tip of snout. Enlargement of drawing—×7.

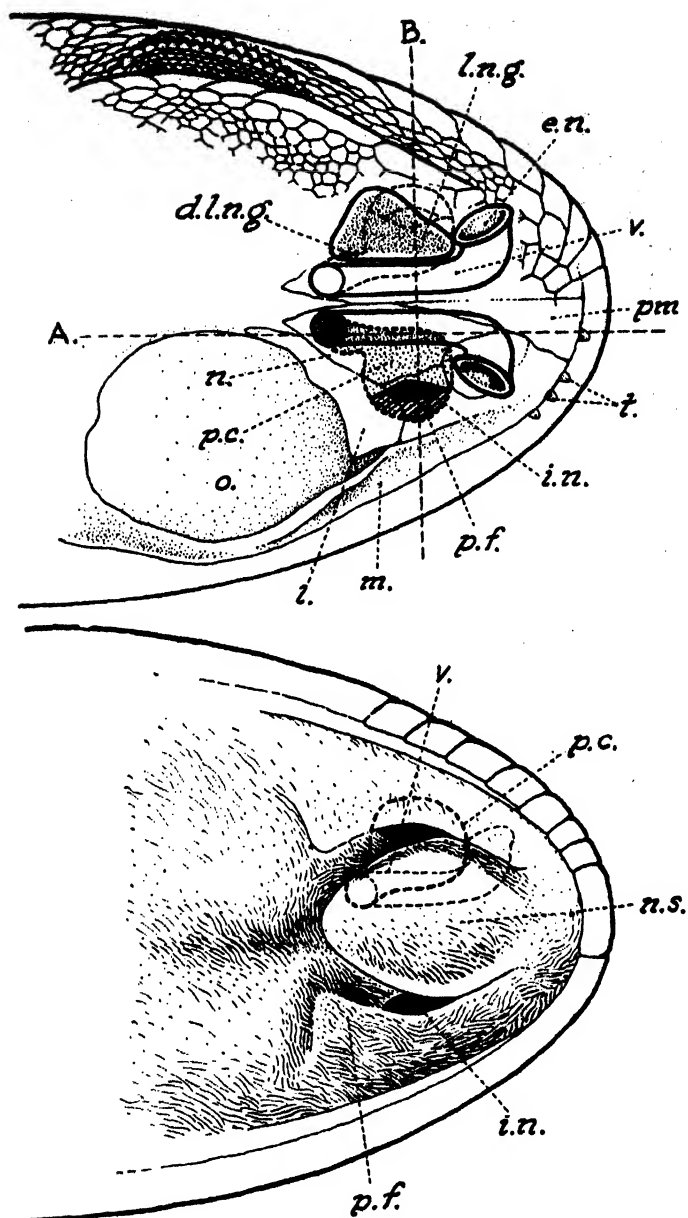|	
c.s.	—ciliated surfaces
d.l.n.g.	—duct of the lateral nasal gland
e.n.	—external naris
i.	—integument.
i.n.	—internal naris
l.	—lacrimal bone
l.n.g.	—lateral nasal gland
m.	—maxillary bone
n.	—nasal bone
n.s.	—nasal septum
n.v.	—nasal valve
o.	—orbit
pm.	—premaxillary bone
p.f.	—palatine fold
p.c.	—principal cavity
t.	—teeth
v.	—vestibule

PLATE I

FIG. 1. Dorsal aspect of the head of *Uma inornata* showing, to the right of the mid-line, the relationship of the olfactory passages to the bones of the nasal region and to the left, the relationship of the nasal structure to the external morphology of the head. The lateral nasal gland has been omitted from the right side.

FIG. 2. Ventral view of the head of *Uma inornata* with the lower jaw removed. Dotted lines indicate the position of the nasal passage on one side.
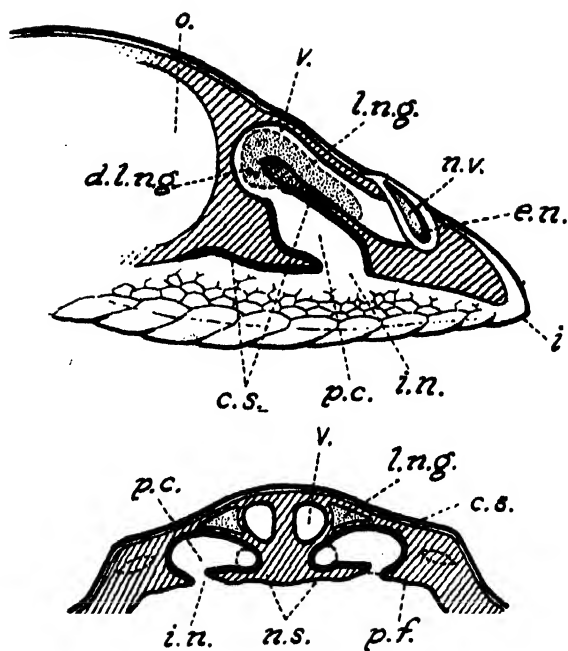
PLATE II

Fig. 1. Semi-diagrammatic parasagittal section through the nasal region of *Uma inornata* (see plate I, fig. 1, A). Heavy lines indicate ciliated surfaces. The drawing has been made to include the internal naris.

Fig. 2. Transverse section through the olfactory organs of *Uma inornata* (see plate I, fig. 1, B). Ciliated surfaces are indicated by heavy lines.

AN ESTIMATE OF THE MINIMUM NUMBER OF GENES DIFFERENTIATING TWO SPECIES OF GOLDEN-ROD WITH RESPECT TO THEIR MORPHOLOGICAL CHARACTERS

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In the recent genetic analysis of evolution and speciation one relevant question of considerable interest from a taxonomic point of view seems to remain unanswered: in how many gene loci, on the average, are lines of descent differentiated at the stage when they are recognized as different species within a single genus? Theoretical studies of species formation have been focussed mainly on the kinetics of establishment, or rejection, of new genes at a *single locus* or within the set of loci relevant to a *single character*. Observational genetic studies of species differences have been mainly chromosomal, or concerned with proving that the visible differences have a mendelian basis, or with ascertaining the general type of gene action involved.

It also would be desirable to know, for at least a few pairs of species, about how many gene substitutions determine the *whole set of differentiating characters* recognized by taxonomists. It is likely that no very reliable answer can be obtained by present methods; and it is to be expected that no two species would give the same answer. However, these probable limitations can scarcely be evaluated or corrected without preliminary, crude attempts to estimate the number of taxonomically important gene differences between particular species pairs. Such an attempt is developed—incompletely—in the following pages, for two golden-rod species.

MATERIAL

Solidago rugosa Mill. is a meadow golden-rod with spreading inflorescence and inconspicuous basal leaf

rosette; *S. sempervirens* L. is a salt-marsh species, shorter than *rugosa,* with compact, secund inflorescence and conspicuous rosette. A specimen of each is shown

S. RUGOSA

SEPT. 21, 1935

S. SEMPERVIRENS   F. HYBRID
                                   S. RUGOSA ♀

Fig. 1.

in Fig. 1. The differentiating characters are compiled in Table 1, taken from Goodwin (1937a).

The two species are not altogether isolated genetically. Where their ranges overlap—as in the reclaimed salt-

marsh near Cambridge, Mass., studied by Goodwin—a variety of intergrades is found. The intergrades can be duplicated in first- and second-generation artificial hy-

TABLE 1

MORPHOLOGICAL CHARACTERS DIFFERENTIATING *Solidago rugosa* FROM *S. sempervirens* (FROM GOODWIN, 1937a)[a]

	S. rugosa[b]	F₁ hybrid	*S. sempervirens*[b]
Natural habitat	Meadows, pastures, and roadsides	Dry borders of salt marshes, etc.	Beaches and salt marshes
Stems (at maturity)			
Height	(75–120) cm	(75–120) cm	(45–90) cm
Pubescence	Thick	Sparse	Absent
Cortex	Not fleshy	Not fleshy	Fleshy
Anatomy	Woody	Woody	Semi-herbaceous
Leaf traces	3	3	7–13[c]
Stolons	Long, spreading	Intermediate	Short, ascending
Leaves			
Length in rosette[d]	(5–12) cm	(10–35) cm	(15–60) cm
Shape	Ovate-lanceolate Narrowed at base Double-serrate	Lanceolate Intermediate Slightly serrate	Oblanceolate Base clasping Entire
Venation	Sub-pinnate	Intermediate	Sub-parallel
Texture	Not fleshy, veins prominent	Intermediate	Fleshy, veins not prominent
Hairs	Numerous, many-celled, linear, persistent; some flask-shaped hairs also present	Both types of hairs present; linear hairs scattered	3-celled, flask-shaped, deciduous (short, curved hairs sometimes on margin)
Length of stomatal apparatus	15 (10–24) μ	22 (15–29) μ	34 (25–46) μ
Cuticle	Not sculptured	Intermediate	Sculptured
Inflorescence			
Panicle	Racemes spreading, panicle broad	Intermediate and variable	Racemes short, panicle contracted
Head			
Involucre length	3.5 (3–4) mm	5.0 (3.6–5.5) mm	5.5 (4–6) mm
Bracts	Scarious except for midvein	Intermediate	Scarious only at margins
No. ray florets	8.5 (6–13)	10.5 (6–14)	11.5 (7–15)
No. disc florets	5.5 (3–7)	10.0 (7–16)	14.5 (11–20)
Total florets	14.0 (12–20)	20.5 (13–29)	26.0 (20–33)
% ray florets	61%	51%	44%
Ray floret length	(3.2–4.0) mm	(4.2–6.5) mm	(8.2–9.0) mm
Disc floret length	(3.0–3.5) mm	(3.0–4.5) mm	(5.0–5.7) mm
Pappus length	(2.0–2.8) mm	(3.0–4.2) mm	(4.5) mm
Fruit length	1.3 (1.0–2.0) mm	2.2 (1.5–2.6) mm	2.9 (2.0–3.6) mm
Fruit weight	0.13 mg	0.27 mg	0.80 mg

[a] Measurements were taken from numerous representative wild plants as well as from plants grown in the greenhouse. Figures represent the modes of each series of measurements. The extremes are indicated in parentheses.
[b] Varieties are not included.
[c] Excluding the cotyledons, which have three traces.
[d] Excluding the first eight seedling leaves and the early spring leaves of the older rosettes.

brids, as Goodwin showed. This indicates a very close relationship between these species, which some investigators may even prefer to reduce to subspecific rank.

The two species appear to be distinct, beyond the narrow range-overlaps, although no adequate study has been made of the extent to which the genom of one has been penetrated by genes of the other. Cytologically the two species seem to be quite alike: each has a haploid chromosome number of nine; the F_1 undergoes normal meiosis and forms normally viable pollen, although the germination of F_1 seeds is only three quarters as good as that of either parent (68 per cent against 90 per cent). Perhaps the "salt-marsh-physiology" genes are so intermingled throughout the chromosomes with *"sempervirens*-morphology" genes—and meadow-physiology with *rugosa*-morphology—that in either habitat the alien structure is continuously eliminated as a by-product of selection against alien function, the elimination balancing the cross-pollination in and near the zones of overlap. In any case, the number of places where the two species grow close enough together to render cross pollination probable are few and far between.

In establishing that the intergrades are natural hybrids, Goodwin (1937a) measured or classified 48 *rugosa*, 38 *sempervirens*, 56 F_1 and 108 F_2 plants, all greenhouse grown, with respect to six of the seven differentiating leaf characters. The results are shown in Table 2.[1]

CALCULATIONS

Genes acting on measured characters. The number of gene substitutions which determine a difference in some measurable character between two isogenic strains can be readily estimated (Wright, 1934) if numerous individuals of each strain and of their F_1 and F_2 have been measured with respect to that character. The method is indicated by the following formula, in which v_P and v_p represent the average measurements of parent strains, σ_1 and σ_2

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[1] Of the F_1 plants, 20 have been omitted from the character 4 and 6 tallies because they were a year older than any of the other plants at the time of measurement; they have been included, however, in the tallies of the other four characters which are thought not to change with age of plant.

TABLE 2

PRINCIPAL FOLIAGE CHARACTERS IN *Solidago sempervirens, S. rugosa* AND THEIR HYBRIDS

Character 1 — leaf margin (entire: 1, 2, 3; serrate: 4, 5)

Category	1	2	3	4	5
sempervirens	38				
rugosa					47
F_1		3	53	1	
F_2	14	31	60	3	

Character 2 — leaf and stem surface (glabrous: 1, 2, 3; pubescent: 4, 5)

Category	1	2	3	4	5
sempervirens	38				
rugosa				8	48
F_1		3	53	2	1
F_2	12	15	71	3	2

Character 3 — leaf thickness (fleshy: 1, 2, 3; not fleshy: 4, 5)

Category	1	2	3	4	5
sempervirens	38				
rugosa					48
F_1	13	3	53	3	2
F_2	6	31	59	11	5

Character 5 — leaf cuticle (sculptured: 1, 2, 3; not sculptured: 4, 5)

Category	1	2	3	4	5	mean	standard deviation
sempervirens	34	4				17.8	...
rugosa				3	45	5.5±	2.02
F_1		5	46	5		10.1	
F_2	4	15	61	18	10	8.2	2.58

Character 4: length of basal leaves

Length (cm)	4–5	6–7	8–9	10–11	12–13	14–15	16–17	18–19	20–21	22–23	24–25	26–27
sempervirens	40											
rugosa						1	3	11	16	17		
F_1			2	5	11	20	16					
F_2			2	11	20	14	9	10	26	15		

Character 6: length of stomatal apparatus

Length (μ)	14–15	16–17	18–19	20–21	22–23	24–25	26–27	28–29	30–31	32–33	34–35	36–37	38–	mean	standard deviation
sempervirens								1	4	8	22	9	2	34.4	2.49
rugosa	23	22	10			1	15	1						15.4	1.82
F_1			9	2	12									21.1	1.07
F_2		3	12				13							23.6	4.16

the standard deviations of F_1 and F_2, and n the number of gene differences:

$$n = \frac{(v_P - v_p)^2}{8(\sigma_2^2 - \sigma_1^2)}$$

For example, the average length of stomatal apparatus in *S. rugosa* is 15.4 μ, in *S. sempervirens* 34.4 μ. The standard deviations of F_1 and F_2 are 1.07 μ and 4.16 μ. So the estimated number of gene differences relevant to stoma length, between the two species, is $(34.4 - 15.4)^2 / 8(4.16^2 - 1.07^2)$ or 3, to the nearest integer.

Wright's formula gives a *minimal* estimate, as he points out. It is derived on the assumptions that (1) each strain is isogenic with respect to the character studied, (2) the large-size genes are concentrated in one strain, the small-size genes in the other, (3) the genes involved have equal effects, without dominance or epistasis, and (4) no two of the loci are in the same chromosome. If these assumptions do not match the actual conditions in material to which the method is applied, the estimated number of gene differences will be smaller than the actual number. To what extent the assumptions are valid in the present case will be discussed below; whatever the answer may be, we shall at least have a minimal estimate.

Genes acting on categorized characters. Wright's method can also be applied to such characters as 1, 2, 3 and 5 (Table 2) with respect to which individuals have been grouped into broad categories, rather than measured (Wright, 1934). For this purpose it is assumed that successive categories represent successive ranges, not necessarily equal, of a normal distribution of values in any particular generation. The value of the boundary between two contiguous categories can then be obtained, in terms of the mean and standard deviation of the generation, by entering a table of areas under the normal curve with the proportion of the group in all categories above (or below) the desired boundary. As an example we may go in detail through the analysis of character 5, degree of sculpturing of leaf epidermis.

numerical value of boundary, T, between categories

	1 and 2	2 and 3	3 and 4	4 and 5
	$T_{1.2}$	$T_{2.3}$	$T_{3.4}$	$T_{4.5}$
sempervirens	$v_s + 1.25\sigma_s$			
rugosa				$v_r - 1.54\sigma_r$
F_1		$v_1 - 1.46\sigma_1$	$v_1 + 1.24\sigma_1$	
F_2	$v_2 - 1.78\sigma_2$	$v_2 - 0.93\sigma_2$	$v_2 + 0.64\sigma_2$	$v_2 + 1.32\sigma_2$

Hence $(v_r - v_s) = (T_{4.5} - T_{1.2}) + 1.25\sigma_s + 1.54\sigma_r = (T_{4.5} - T_{1.2}) + 2.79\sigma_1$

If $\sigma_r = \sigma_s = \sigma_1$

$(T_{3.4} - T_{2.3}) = (1.24 + 1.46)\sigma_1 = (0.64 + 0.93)\sigma_2$, so $\sigma_2 = \dfrac{2.70}{1.67}\sigma_1 = 1.62\sigma_1$

$(T_{4.5} - T_{1.2}) = (1.32 + 1.78)\sigma_2 = 3.10\sigma_2 = 3.10 \times 1.62\sigma_1 = 5.01\sigma_1$

so $(v_r - v_s) = (2.79 + 5.01)\sigma_1 = 7.80\sigma_1$, and

$$n = \frac{7.80^2\sigma_1^2}{8(1.62^2 - 1^2)\sigma_1^2} = 5 \quad \text{(to the nearest integer)}$$

The same method, with one modification, can be applied to the remaining categorized characters: where there are individuals on only one side of a boundary whose value is essential (*e.g.*, character 2, F_1, 3–4 boundary) it is assumed that the boundary is 2.5 σ from the group mean.

Total gene differences relevant to leaf characters. The significant statistics for each of the six leaf characters recognized as differentiating *S. sempervirens* from *S. rugosa*, and the corresponding estimates of gene differences, are shown in Table 3.

TABLE 3

Statistics for Determination of Minimal Total Gene Differences, Relevant to Leaf Characters, Between *Solidago sempervirens* and *S. rugosa*. Δ is the Difference in Average Measurement Between Species Divided by the Standard Deviation of F_1; R is the Standard Deviation of F_2 Divided by that of F_1; $N = \Delta^2/8(R^2 - 1)$, to the Next Integer Above the Exact Value

Character	Δ	R	Minimal number of gene differences n	
1. leaf margin: entire to serrate	11.0	1.81	7	6.6)
2. leaf surface: glabrous to pubescent ...	11.8	2.06	6	5.2)
3. leaf thickness	11.7	2.06	6	5.3)
4. basal leaves: length	6.1	1.28	8	7.4)
5. leaf cuticle: degree of sculpturing	7.8	1.62	5	4.7)
6. stomatal apparatus: length	17.8	3.89	3	2.6)
		Total	35	

It is of some interest to note that here, as in so many previous and more reliably studied cases, even the probably unimportant morphological details such as serration

of leaf margin are controlled by genes in many or most of the chromosomes. Of course, only a very small part of the genetic control of each character is detected, namely, that part which is unlike between two species which are certainly very closely related.

The *total* leaf-differentiating loci need not be as numerous as 35, the total of Table 3. Length and thickness of leaves and size of stomatal apparatus are all "size characters," and so are perhaps determined to some extent by the same genes. Each gene with effects on more than one of the characters studied would contribute to the F_2 variation of each character, and so would be included in Table 3, once for each effect.

How many genes with manifold effects are represented here can be estimated by a method which involves assumptions 1, 3 and 4 of Wright's formula (*cf.* page 58 of this paper). The following quantities are used:

Δ_x, the difference between parents in average value of character x, divided by the standard deviation of that character in F_1, *i.e.*, $\Delta_x = (x_P - x_P)/\sigma_{x1}$;

Δ_y, the analogous quotient for character y, *i.e.*, $\Delta_y = (y_P - y_P)/\sigma_{y1}$;

R_x, the quotient of standard deviations of character x in F_1 and F_2, *i.e.*, $R_x = \sigma_{x2}/\sigma_{x1}$;

R_y, the analogous character-y quotient, *i.e.*, $R_y = \sigma_{y2}/\sigma_{y1}$;

r_1, the correlation between characters x and y in F_1;

r_2, the correlation in F_2;

n_0, the number of genes acting on both characters;

n_L, the number of character-x loci each linked with a character-y locus;

c, the average crossover value between linked pairs of genes.

This method leads to the following formula:

$$n_L(1 - 2c) + n_C = \frac{\Delta_x \Delta_y (r_2 R_x R_y - r_1)}{8(R_x^2 - 1)(R_y^2 - 1)}$$

The quantities in the right-hand member of the equation, except the correlation coefficients, are those of Table 3. The correlation coefficients, calculated from the data, are taken as positive, whatever their actual sign may be.

For length of leaves and of stomatal apparatus

$$n_L(1 - 2c) + n_C = \frac{6.1 \times 17.8(0.348 \times 1.28 \times 3.89 - 0.183)}{8(1.28^2 - 1)(3.89^2 - 1)} = 2.33$$

Therefore it seems relatively improbable that all three stoma genes also affect leaf size, since n_C could not be 3

unless $n_L(1-2c)$ were negative, which is meaningless. Three possibilities are left, any of which satisfies the condition that there are three stoma gene differences altogether:

Number of common genes	Number of linked pairs	Average crossover value (c)	Total stoma and leaf length genes
2	1	33%	9
1	2	17%	10
0	3	11%	11

Which of these possibilities is correct can not be determined, since they all meet the only available condition, that $n_L(1-2c)+n_c$ should have a value of 2.33. Now if it is recalled that 3 is a *minimal* estimate of stoma-length genes, and 8 a *minimal* estimate of leaf-length genes, it becomes obvious that the comparable limiting value for leaf and stoma jointly is that of *maximal common genes*, 2, and of *minimal total genes*, $3+8-2=9$. In general, then, the possibility of linked gene pairs need not be considered further, and attention can be confined to common genes, of which the maximal estimate is the next integer below the value of $n_L(1-2c)+n_c$ for any particular character pair.

The calculated values of $n_L(1-2c)+n_c$ for each of the fifteen pairs among the six leaf characters studied are shown in Table 4, with the correlation coefficients upon which they depend. The correlation coefficients were calculated from the original data (unpublished). The maximal common gene estimates are shown in Fig. 2.

The interrelationship, shown by Fig. 2, within the genom which is responsible for the distinguishable leaf differences between *Solidago sempervirens* and *S. rugosa* might seem almost hopelessly complicated. But in fact an answer is readily obtained to the one question under consideration here: what is the *smallest* number of gene substitutions which will account for the phenotypic differences? No two among the characters 1, 4 and 5 show any evidence of common genes (common gene indices,

in Table 4, 0–1; estimates of maximal common genes, in Fig. 2, 0). Hence the differences in serration, length and sculpturing altogether *can not* be accounted for by a number of gene differences less than the sum of the substitutions affecting the characters separately, *i.e.,* 7 + 8 + 5 or 20.
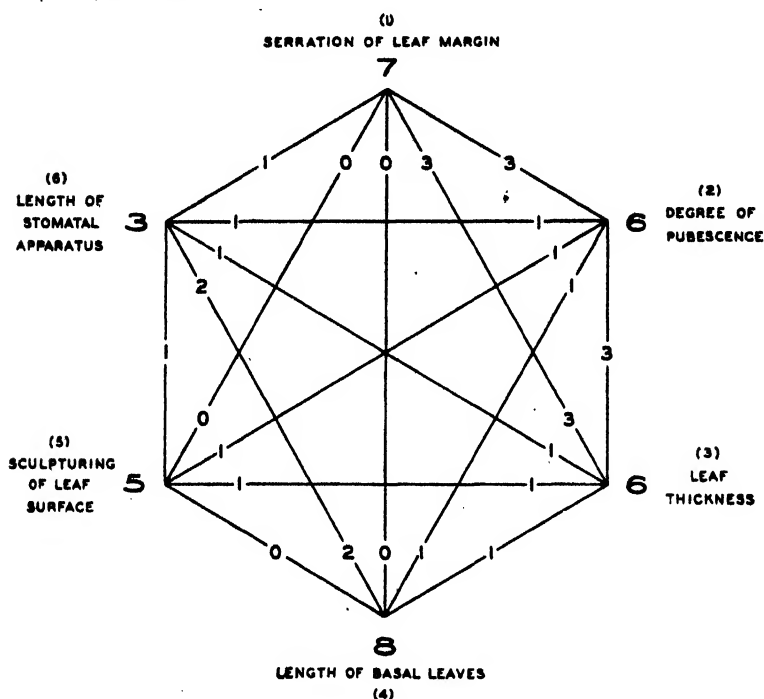
FIG. 2. Leaf characters differentiating *Solidago sempervirens* from *S. rugosa*, estimated minimal number of gene differences determining each character difference (large digits at apices of hexagon), and maximal number of genes acting on both members of each possible character pair (small digits on lines connecting characters).

Some among the 20 might also account for the stoma-length difference, since Fig. 2 shows that stoma length and epidermal sculpturing may be affected in common by one gene, stoma and leaf length by two, stoma length and marginal serration by one. The potential total, four, is greater than the number of stoma genes to be accounted for, three. Of course it is possible that one or more of the stoma length genes is in fact different from any of the 20

which control characters 1, 4 and 5. In this case the total controlling the differences in characters 1, 4, 5 and 6 altogether would be greater than 20, which is the least possible total.

Character 3, leaf thickness, may be affected in common with leaf serration by as many as three genes; with epidermal sculpturing, by one; with leaf length, by one. The potential maximum of 5 genes in common with the pre-

TABLE 4

Correlations between pairs of taxonomically significant leaf characters in F_1 and F_2 hybrids between *Solidago sempervirens* and *S. rugosa*, with an index which is equal to the number of genes acting on both characters *plus* a function of the number of linked gene pairs (one gene of each pair affecting one character, one the other). The characters involved are: 1, serration of leaf margin; 2, degree of pubescence; 3, thickness; 4, length of basal leaves; 5, sculpturing of leaf surface; 6, length of stomatal apparatus.

| Character pair | Correlation* in | | Common gene index |
	F_1	F_2	
1.2	+.071	+.448	3.5
1.3	−.008	+.457	3.7
1.4	−.087	−.066	0.4
1.5	0	+.038	0.3
1.6	+.066	−.271	1.4
2.3	+.068	+.514	3.5
2.4	−.059	−.163	1.6
2.5	0	+.199	1.5
2.6	+.028	−.272	1.2
3.4	−.067	−.176	1.7
3.5	0	+.136	1.0
3.6	+.136	−.399	1.7
4.5	−.103	−.073	0.3
4.6	+.183	+.348	2.4
5.6	−.101	−.350	1.6

* In calculating the coefficients which involve characters 1, 2, 3 and 5 the class values of Table 2 were transformed as in Pearson's broad categories correlation method. *But* the standard deviations of the categorized characters were taken as one rather than the quantity given by the transformed class values. This procedure was followed because the F_1 data are so scanty that the calculated standard deviations are in some cases considerably affected by the classification of one or a few individual plants. The procedure underestimates the true correlations, more so in the F_1 than in the F_2; the errors in the two generations have opposite, and so at least partly compensating, effects on the value of the common gene index.

viously considered characters leaves one of the six thickness genes unaccounted for. So the total phenotypic differences in characters 1, 3, 4, 5 and 6 altogether must depend on at least 20 plus 1 gene substitutions. These 21 will also account for the genetic control of differences in pubescence which may have three genes in common with serration, one with sculpturing, one with leaf length and three with leaf thickness.

Altogether then, the six distinguishable leaf differences

between *Solidago sempervirens* and *S. rugosa* might be determined by as few as 21 gene substitutions. That the estimation method leaves open the possibility—indeed, the probability—of more than 21 should perhaps be recalled here. But there can scarcely be fewer. A number less than 21 would not account for the observed differences in standard deviations and correlations between F_1 and F_2 from which the estimates of Table 4 and Fig. 2 are derived.

In criticism of this conclusion it might well be pointed out that the statistics upon which the estimates depend, and therefore the estimates themselves, are subject to large sampling errors because the populations measured were so small. Furthermore, the estimation methods are strictly applicable only to crosses between isogenic strains, while the P_1 plants of the present crosses were grown from the seeds of wild plants or were only one generation removed from wild seeds. To these criticisms no answer seems possible except that suggested above, that a very crude attempt at the problem is perhaps better than none at all.

The criticisms might arise that the estimations are aimed at the minimal, rather than the most probable, number of gene differences, at readily recognizable phenotypic contrasts, rather than the whole array; and that no consideration is given to the inevitable complex linkage relations within a set of 21 loci distributed among 9 chromosomes. The answer here is obviously one of what is practicable without endless mendelian and physiological study.

An estimate of total gene differences relevant to all the morphological characters. A minimum estimate has now been made of the number of genes involved in differentiating *S. sempervirens* from *S. rugosa* with respect to six obvious morphological leaf characters. The question posed earlier in this paper as to what may be the minimum number of genes producing all the obvious morphological differences between these two species still

remains unanswered. Reference to Table 1 will show that other characters may be found in the stems, leaves and inflorescence. To what extent would an analysis of these other characters be likely to increase our minimum estimate of gene number?

It is well known that certain genes may have a general effect upon the development of several different parts of an organism, as in the case of a number of genes in *Primula sinensis* (de Winton and Haldane, 1933). In a recent analysis of the specific differences between two species of *Nicotiana,* Anderson and Ownbey (1939) have pointed out that these differences may be grouped into categories which they call genetic coefficients. The inference is that a single gene or a battery of genes controlling the expression of a morphological character in one structure may control the development of a similar character in another type of structure in the same plant. Differences in one or more genes within such a battery might then account for morphological differences in various parts of the plants of the two related species. In *Nicotiana* crosses, for example, Anderson (1939) reports a close positive correlation between the length of style and the magnitude of various corolla measurements. There is no certainty, however, that a given set of genes will always have similar morphological effects in all parts of an organism. Indeed, this has been shown not to be the case in certain instances.

Regardless of this criticism, in order to make an estimate of the *minimum* number of genes differentiating the golden-rod species under consideration, this principle of "genetic coefficients" should be applied. If the assumption should prove to be incorrect in any particular case it would mean increasing our estimate. Hence the various characters in Table 1 have been regrouped under the following headings according to their possible genetic association and the nature of the association is discussed briefly in each case.

(1) *Organ size.* In general, most of the organs of *S.*

sempervirens are larger than those of *S. rugosa*. A developmental analysis of the leaves of these two species (Goodwin, 1937b) has shown that greater cell number and greater cell size in the lamina and along the midrib, and prolonged meristematic activity near the base of the petiole, are responsible for the larger total size and longer petiole of *S. sempervirens*. Hence the genetic control of leaf size (and at the same time of leaf shape) must be most complex in this material. The previous analysis of character 4, leaf length, indicates that at least 8 genes are contributing toward the differentiation of the two species with respect to this character, while size differences in the stomatal apparatus, character 6, are controlled by at least three genes not more than two of which are common to the leaf-size set.

In the inflorescence, all the floral parts are also more or less proportionately larger in *S. sempervirens* than in *S. rugosa;* involucral bracts, receptacle, ray florets, disc florets, pappus, fruit, etc. The total number of florets is probably dependent upon the size of the receptacle; and the proportion of ray to disc florets may also depend upon receptacle size, since ray florets are in a marginal position in the head and since the margin of the receptacle increases directly with its diameter, while the area increases as its square. In estimating the minimal number of genes involved, the assumption might be made that the same set of genes operates both in the leaves and in the reproductive structures in determining these size differences.

(2) *Length of axes.* The length of the main axis of the shoot, of the lateral branches of the inflorescence, and of the underground stolons is greater in *S. rugosa* than it is in *S. sempervirens*. These are the only structures which are larger in this species. No genetic data are available on this series of size characters. It is entirely possible that they are all differentiated at least in part by a common set of genes.

(3) *Parenchyma.* The stem cortex and leaf mesophyll

are developed to a greater extent in *S. sempervirens* than they are in *S. rugosa*. This gives both of these structures a more fleshy, succulent appearance in the former species. The genetic analysis of leaf thickness, character 3, indicates that at least 6 genes are involved. These may also influence the development of the cortex. It is suggested that this same set of genes may be responsible for the lack of scarious margins in the bracts of *S. sempervirens* as well.

(4) *Leaf traces.* In *S. rugosa* and the F_1 hybrid the leaf traces are three in number. In *S. sempervirens* the number ranges from 7 to 13. The leaf traces connecting the vascular system of the stem with that of the leaf leave gaps in the vascular cylinder above their point of departure. The large number of these gaps in *S. sempervirens* contributes toward the herbaceous nature of the stem in this species. Furthermore, the traces going to a given leaf arise from points all around the stem and pass directly into the clasping base of the petiole as parallel veins. No genetic analysis of leaf trace determination has been made. The nature of the stem, the leaf base and the leaf venation might all be characters affected by this factor.

(5) *Leaf margin.* The leaves of *S. rugosa* are serrate while those of *S. sempervirens* are entire. The nature of the leaf margin, character 1, appears to be controlled by at least 7 genes.

(6) *Pubescence.* Epidermal hairs are of two sorts in these species. Linear, multicellular hairs are present in *S. rugosa,* but not in *S. sempervirens*. Six genes are apparently involved in determining this character difference. Flask-shaped, deciduous hairs are present in *S. sempervirens,* but not in *S. rugosa*. In this case no genetic analysis is available. The character is not of much practical importance to the taxonomist, since these hairs are usually absent from mature leaves and from herbarium specimens.

(7) *Cuticular surface.* In the leaves of *S. semper-*

virens the cuticle is sculptured into many close furrows, whereas the cuticle of *S. rugosa* is smooth. In this case, character 5, our analysis indicates a minimum of 5 genes in operation.

It has already been estimated that a minimum of 21 genes is responsible for the morphological differences between the leaves of *S. rugosa* and *S. sempervirens,* a battery of genes being responsible for the differentiation of each character. The foregoing discussion lays the basis for the assumption that certain other characters in the stem and inflorescence may be differentiated by these same batteries. Characters which may well be differentiated by sets of genes different from and comparable in number to any included in our previous estimate are the length of the axes, the number of leaf traces and the presence of flask-shaped hairs. Hence it might be legitimate to increase our estimate by a factor of two, giving us about 40 gene substitutions responsible for all the obvious morphological differences between these two species. Any genes common to the unstudied characters and to the leaf characters would reduce this figure. We realize that this figure is at best a very crude minimum estimate. It should be pointed out again that the actual number of genes involved is probably much larger than this minimum value.

A similar but more exhaustive analysis on more suitable genetic material such as the *Nicotiana Langsdorffii-N. alata* cross, which has already been studied in considerable detail, is greatly to be desired.

Summary

The differences between the leaf characters of two species of golden-rod, *Solidago rugosa* and *S. sempervirens,* have been shown to be determined by a minimum of 21 genes. It is pointed out that a minimum of about twice as many genes are probably in operation in the control of all the morphologically distinguishable differences between these two species.

A method has been devised to estimate the maximum number of gene differences common to two morphological character differences between related strains or inter-fertile species from the standard deviations and correlations of the character pairs in the F_1 and F_2 hybrids.

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THE LINKAGE OF POLYDACTYLY WITH MULTIPLE SPURS AND DUPLEX COMB IN THE FOWL[1]

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FOLLOWING discovery of a fifth autosomal linkage group in the fowl, containing the genes, D, for duplex comb, and M, for multiple spurs (Hutt, 1941), further tests were made for linkage of other genes with these two.

LINKAGE OF M AND Po

The studies already reported showed M to be independent of the other four autosomal linkage groups, and it was therefore desired to test it and D with other mutations known not to belong to those groups. A test of four such genes was easily arranged by mating black $dd\ MM$ females with a Mottled Houdan male and backcrossing to Anconas. The F_1 heterozygotes were of the constitution $\dfrac{D\ m\ Cr\ Mu\ Po\ W\ e'}{d\ M\ cr\ mu\ po\ w\ E'}$ carrying the four mutations, Mu (muffs), Po (polydactyly), W (white skin) and e' (mottling), which had not been assigned to any known linkage group, although all but mottling had been used in several linkage tests. These birds were all heterozygous for crest (Cr), which was not needed in this study because M had already been proven independent of the linkage group $(F\ I\ Cr)$ to which crest belongs. Some of these linkage testers also carried the gene Fl (flightless) previously shown to be not linked with M.

When the multiple heterozygotes were backcrossed to Anconas, which carry the recessive alleles of all the mutations mentioned above, Mu, W and e' showed independent

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assortment with *D* and *M* and with each other. However, *Po* proved to be linked with *m*, there being 33.5 per cent. crossing over in 514 gametes.

Polydactyly is not an ideal mutation for linkage studies because it is suppressed in many heterozygotes. Backcrosses yielding clear 1 : 1 ratios are not usual. Because polydactyly has previously been assigned by Dunn (1927) and Dunn and Landauer (1930) to the *F I Cr* chromosome, and also because it has been used in many unsuccessful tests for linkage, the present evidence for its linkage with *M* and *D* is given in full. This comes mostly from progeny of ten females mated to an Ancona male, H 5100, in the spring of 1941. Classifications of these chicks at hatching are shown in Table 1. In most cases these were verified at later ages, although this was not essential because polydactyly is easily recognized in the day-old chick, and, as was previously shown (Hutt, 1941), classifications of multiple spurs at that age are almost completely accurate.

TABLE 1

EVIDENCE FROM BACKCROSSES THAT POLYDACTYLY, *Po*, IS LINKED WITH
MULTIPLE SPURS, *M*

Heterozygotes tested	Classification of progeny				Gametes tested		Crossing over
	Parental classes		Crossovers		Total	Crossovers	
	Po m	*po M*	*Po M*	*po m*	*number*	*number*	*per cent.*
♀ 4251	19	31	12	16	78	28	35.9
4257	26	23	13	16	78	29	37.2
4259	8	13	8	8	37	16	43.2
4261	35	24	11	14	84	25	29.8
4262	16	23	9	9	57	18	31.6
4272	23	24	10	15	72	25	34.7
4255	11	7	4	6	28	10	35.7
4260	13	10	9	2	34	11	32.4
4263	9	6	1	2	18	3	16.7
4266	13	8	1	6	28	7	25.0
Total	173	169	78	94	514	172	33.5

In the 514 birds from this mating, the ratio of *Po* : *po* was 251 : 263, while that of *M* to *m* was 247 : 267. Expressed otherwise, polydactyly was present in 98 per cent. of the number expected (257) and multiple spurs in 96 per cent. Since the deviation from expectation was so small with both mutations, it follows that there was little or no suppression of *Po* or *M*, and hence that the data are

suitable for the measurement of linkage. The composite
figure of 33.5 per cent. crossing over is reasonably re-
liable, not merely because it was measured in 514 gametes,
but also because these came from ten different birds,
among which some variability in the amount of crossing
over was to be expected. This figure was substantiated
by another backcross in which a male heterozygous for
M and *Po* was mated with doubly recessive females.
Sixty per cent. of the 98 progeny were eliminated from
consideration because manifestation of *Po* in their sib-
ships was considerably below the expected 50 per cent.
However, in the remaining 39 chicks, comprising families
with little or no suppression of *Po,* the number of new
combinations was 14. The amount of crossing over in
this male—35.9 per cent.—was therefore quite close to the
33.5 per cent. measured in gametes of the ten females
listed in Table 1.

Linkage of *Po* Obscured by Its Suppression

These studies provided a good example of the way
in which linkage tests involving polydactyly (or other
genes) might be incorrectly interpreted whenever the
character is not manifested in all heterozygotes. The
last four females listed in Table 1 were originally bred to
another Ancona male, H 4999, that apparently carried
modifying genes suppressing both *Po* and *M.* In his
118 progeny, the ratio of *Po* to *po* was 24 : 94, and of
M to *m,* 34 : 84. With 59 expected in each class, it is
clear that polydactyly was suppressed in about 60 per
cent. of the birds heterozygous for it, and multiple spurs
in 42 per cent. In striking contrast to this, the mating
of these same four hens with ♂ H 5100 yielded an excess
of polydactylous offspring above the 50 per cent. expected
and a slight deficiency of multiple-spurred birds no
greater than could have occurred by chance.

These diallel crosses show nicely (1) the difficulty of
measuring linkage in matings where *Po* is suppressed and
(2) the validity of linkage tests where manifestation of

TABLE 2
DEMONSTRATION BY DIALLEL CROSSES OF DISTORTED RATIOS AND CONCEALMENT OF
LINKAGE CAUSED BY SUPPRESSION OF TWO LINKED CHARACTERS

Mating of four females	Classification of progeny				Gametes tested	Crossing over
	Parental combinations		New combinations			
	Po m	*po M*	*Po M*	*po m*	*number*	*per cent.*
(a) With ♂ H 5100	46	31	15	16	108	28.7
(b) With ♂ H 4999	21	31	3	63	118	55.9
Expected with 33.5 per cent. crossing over*	10.4	20.4	4.6	64.6	118	33.5
Expected with no linkage* .	17.1	27.1	6.9	66.9	118	none
Expected with no linkage and no suppression	29.5	29.5	29.5	29.5	118	none

* When corrected on the basis that *Po* is suppressed in 59 per cent. and *M*
suppressed in 42 per cent.

that character is not inhibited (Table 2). The mating
with ♂ H 5100 revealed 28.7 per cent. crossing over be-
tween *Po* and *M*, a figure not out of line with the 34.7 per
cent. measured in gametes of the other six hens in the
same mating. In contrast to this, progeny of ♂ H 4999
showed an apparent crossing over of 55.9 per cent., which
would ordinarily be interpreted as indicating the inde-
pendence of *Po* and *M*. Suppression of these two genes
reduced the *Po M* class to 3 and increased the double
recessives to 63. When the expected ratio is calculated
after making allowance for the suppression of *Po* and *M*
and for 33.5 per cent. crossing over (Table 2), a close fit
of the numbers observed to those expected in each class is
obtained ($\chi^2 = 0.85$). Even the deviation of the observed
ratio from that expected with the same allowance for sup-
pression of the two genes but with no linkage is only
slightly greater than could occur by chance ($\chi^2 = 3.91$,
$p = .05$). This is because the expected $1 : 1 : 1$ ratio
is distorted more by suppression of *Po* and *M* than by
crossing over.

It is evident that tests for linkage of *Po* can be utilized
whenever that character segregates in a clear $1 : 1$ or $3 : 1$
ratio but are subject to suspicion when it does not.

ARRANGEMENT OF D, M AND Po IN THE CHROMOSOME

In the previous report, Hutt (1941) showed that D and
M are linked with 28 per cent. crossing over between

them. Since there is 33.5 per cent. crossing over between
M and *Po,* it follows that *Po* must be either to the left of
D, and very close to it, or so remote from *D* as to appear
almost independent. That the latter alternative is the
correct one is shown by the following data for 517 progeny
of the ten females listed in Table 1, each of which carried
D and *Po* in the coupling phase:

Parental combinations		New combinations		Crossing-over
D Po	*d po*	*D po*	*d Po*	per cent.
number	number	number	number	
143	157	108	109	42

Since there was in this population almost complete
manifestation of polydactyly (252 *Po* : 265 *po*) and of
duplex comb (251 *D* : 266 *d*), the 42 per cent. crossing
over can not be questioned. The relationship of the
three genes is therefore approximately *D* 28 *M* 33 *Po.*

The relation of *D* to *Po* had previously been examined
by the senior author, but, since in his (unpublished) data
for 631 gametes tested the manifestation of *Po* was only
59 per cent. of the number expected, the test was of
doubtful value and no conclusions were drawn. (Actu-
ally, when the crossovers between two linked genes ap-
proach 50 per cent., as in this case, the distortion of ratios
by suppression of one of the linked genes causes little
error in the measurement of linkage. This is because
the number of zygotes converted by such suppression
from a parental to a crossover class is almost balanced
by the reciprocal conversion.) Similarly, the data of
Serebrovsky and Petrov (1930), who found 31 per cent.
crossing over between *D* and *Po* in one test, were hardly
conclusive because in two others the amounts of apparent
crossing over were 53 and 54 per cent. In these two
matings, manifestation of one or both of the two dominant
alleles was poor.

Double crossing over. The constitution of ♂ H 5100
with respect to the three linked genes was $\dfrac{D\ m\ Po}{d\ M\ po}$. It
would be expected that, when the cumulative "distance"

between D and Po was 61 per cent., there should be some double crossing over between these two genes. The number of such cases observed was 48, or 9.3 per cent., which, oddly enough, was exactly the number expected $\left(\dfrac{28 \times 33.5}{100}\right)$. Of these, 17 were $D\ M\ Po$ and 31 $d\ m\ po$. The amount of apparent crossing over between D and Po expected on this basis was $61 - (2 \times 9.3)$, or 42.4 per cent., a figure almost identical with the 42 per cent. actually observed.

Modifying Genes

The diallel crosses of four females heterozygous for both M and Po to two different males (Table 2) suggest that modifying genes preventing the appearance of polydactyly in heterozygotes also inhibit the manifestation of multiple spurs. In 108 progeny of H 5100, the proportion with Po was 56 per cent., and with M, 43 per cent., both figures being reasonably close to the expected 50 per cent. In contrast, among 118 progeny of these same females by ♂ H 4999, only 20.3 per cent. exhibited polydactyly and 29 per cent. had multiple spurs.

Similarly, in another mating in which an $Mm\ Po\ po$ male was backcrossed to doubly recessive females, the number of progeny with the dominant allele was only 74 per cent. of that expected for polydactyly and 88 per cent. of the number expected with multiple spurs. Although it seems likely that modifiers suppressing polydactyly tend also to inhibit multiple spurs, the effects are not equal upon both mutations, and different modifying genes may be involved. From previous studies it is clear that polydactyly is suppressed more often than multiple spurs. It is perhaps to be expected that both mutations would be affected by the same modifying genes, since the areas involved are in close proximity and the effects of the two genes are somewhat alike. Po causes duplication of one or more phalanges of the first toe, while M modifies several scales and induces from three to five centers of ossification where only one is normal. Although these

processes are not identical, they have some features in common.

Genes Independent of the *D M Po* Chromosome

For the convenience of other investigators, there is summarized in Table 3 the evidence previously published and some new data to show which genes are independent of this latest linkage group to be discovered in the fowl. Some of the studies with multiple spurs were reported previously. Other tests of *M* with mottling, muffs and beard and white skin are omitted from Table 3 because, since these last three mutations are independent of *D* and

TABLE 3

Characters Apparently Independent of the *D M Po* Group

Linked gene	Tested with	Investigator		Back-cross ratio*
	Cp R U group			
D	Rose comb	Serebrovsky and Petrov	(1930)	61 : 55
"	Creeper	Landauer	(1932)	145 : 141
Po	Creeper	Serebrovsky and Petrov	(1930)	99 : 91
"	Rose comb	" " "	(1930)	417 : 391
"	Rose comb	Warren	(1933)	148 : 146
	F I Cr group			
D	Frizzling	Warren and Hutt	(1936)	172 : 150
"	Crest	Serebrovsky and Petrov	(1930)	225 : 203
"	Crest	Warren and Hutt	(1936)	409 : 389
Po	Frizzling	" " "	(1936)	163 : 166
"	Crest	Serebrovsky and Petrov	(1930)	160 : 130
"	Crest	Warren	(1933)	121 : 113
	O P Ma group			
D	Pea comb	Serebrovsky and Petrov	(1930)	123 : 104
Po	Pea comb	" " "	(1930)	152 : 160
	h Fl group			
D	Flightless	Hutt and Mueller		192 : 183
M	"	Hutt	(1941)	120 : 122
	Other mutations			
D	Blue	Serebrovsky and Petrov	(1930)	47 : 39
"	Recessive white	" " "	(1930)	13 : 13
"	Extension of black	" " "	(1930)	276 : 219
"	Mesodermal pigment	" " "	(1930)	143 : 128
"	Muff and beard	" " "	(1930)	241 : 226
"	Muff and beard	Hutt and Mueller		367 : 344
"	Naked neck	Landauer	(1932)	102 : 104
"	White skin	Serebrovsky and Petrov	(1930)	62 : 61
"	White skin	Hutt and Mueller		138 : 142
"	Mottling	" " "		324 : 328
Po	Blue	Serebrovsky and Petrov	(1930)	162 : 171
"	Extension of black	" " "		250 : 241
"	Feathered legs	Warren	(1933)	199 : 182
"	Mesodermal pigment	Dunn	(1927)	41 : 47*
"	Muff and beard	Serebrovsky and Petrov	(1930)	143 : 144
"	Muff and beard	Hutt and Mueller		345 : 367
"	Naked neck	Serebrovsky and Petrov	(1930)	144 : 182
"	Naked neck	Warren	(1933)	187 : 239
"	Rumpless	"	(1933)	199 : 181
"	White skin	"	(1933)	112 : 110
"	White skin	Hutt and Mueller		150 : 130
"	Mottling	" " "		328 : 326

* Ratios given are those of parental combinations to new combinations in backcrosses, except for Dunn's figures, which are from an F_1 population.

Po, at the opposite ends of the linkage group, they must also be independent of *M* in its center. Similarly, it is unnecessary to give the extensive evidence that *D, M* and *Po* are independent of dominant white so long as these three are shown to be independent of frizzling and crest, which are at opposite ends of the group to which dominant white belongs.

Undated tests ascribed to the present authors refer to data not previously published. Details of the linkage groups mentioned in Table 3 are given by Hutt and Lamoreux (1940). Both *D* and *Po* are independent of one or more genes in the first three groups listed. The fourth one, containing the mutations silky and flightless, has been tested with *D* and *M*, but data of the present writers on the possible linkage of *Fl* and *Po* were not satisfactory because *Po* was suppressed in those matings. From this summary it seems probable that the genes *D, M* and *Po* comprise a separate linkage group, the fifth autosomal one to be found in the fowl. Seven genes not previously assigned to any linkage group are also shown to be independent of both *D* and *Po*.

SUMMARY

Polydactyly was found to be linked with multiple spurs and with duplex comb. The arrangement of these genes and the approximate crossover distances separating them are: *D* 28 *M* 33 *Po*. The amount of crossing over measured between *D* and *Po* was 42 per cent. and there were 9.3 per cent. double cross overs.

These determinations were made in backcross populations showing a normal 1 : 1 ratio of polydactylous to four-toed birds. It is shown that interpretation of linkage tests with *Po* is difficult when, as frequently happens, inhibiting genes prevent the manifestation of polydactyly in heterozygotes. Genes suppressing this mutation seem also to suppress multiple spurs.

A summary is given of published evidence and new data showing that *D* and *Po*, at opposite extremes of this link-

age group, are apparently independent of four other autosomal linkage groups and of seven other genes with which both have been tested.

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REVIEWS AND COMMENTS

EDITED BY CARL L. HUBBS

Race, Reason & Rubbish A Primer of Race Biology. By GUNNAR DAHLBERG. Translated from the Swedish by LANCELOT HOGBEN. New York: Columbia University Press, 1942: 1–240, figs. 1–44. $2.25.

WHILE serving as director of the State Institute of Human Genetics in the University of Uppsala, Gunnar Dahlberg prepared for the Swedish public a rational and studious discussion of one of the hot questions of the day. Lancelot Hogben, feeling that a wider reading of the Dahlberg product would be good medicine for a sick world, has translated the book into what he calls the Anglo-American language.

Dahlberg is convinced that the popular ideas that are rampant to-day regarding human races are largely erroneous, because they are based on prejudice and politics rather than on scientific principles. Consequently he devotes much of his book to a popular and generally excellent presentation of the facts and interpretations of modern genetics. This treatment is marred by some confusing errors in the calculations of Mendelian ratios (on pp. 80, 142 and 144). These slips are attributable, I suppose, to the difficult conditions under which the book was seen through the press.

The basic principles of genetics are then applied to human biology. It is held that through panmixia the ratios of genes in human populations tend to remain con-

stant, except as they are altered by mutation, selection, inbreeding, assortive mating and isolation. The last-named factor, unnecessarily called "the isolate effect," is held to comprise one of the two fundamental questions of human genetics, ranking in significance with selection arising from different fertility in social groups. The recent enlargement of the "isolates" (areas or units of effective breeding populations), through migration to towns, industrialization and increased communication, is regarded as a potent eugenic force. The assigned reason is that defective genes tend to increase in small breeding units, and to decrease in larger "isolates."

It is held that selection, as through sterilization, can not be effective in significantly reducing the incidence of recessive defects, because such characters are rare. Since persons showing the defects are few, a relatively high ratio of unaffected heterozygotes would continue to carry the defects and lead to the production of defective homozygotes in scarcely reduced numbers, even though most of the unfortunate should be prevented by law from reproducing.

Dahlberg's contributions to the understanding of race problems in man are significant and demand attention. Some of his views, however, seem inconsistent, and perhaps too strongly flavored with the social as opposed to the biological viewpoint. Racial differences in anything but outward characters are treated as improbable, because not yet proved to the author's complete satisfaction by objective research. Individual variations, particularly in the European groups, are taken as evidence against race concepts. Dahlberg strongly discounts the generally accepted idea of ethnologists that varying and mixed characteristics in human populations are traceable to the interbreeding of races that were previously more distinct. In this view he seems to have swung as far to one extreme as some anthropologists have gone to the other extreme in assigning members of a single interbreeding population to distinct racial types.

The Vertebrate Eye and Its Adaptive Radiation. By GORDON LYNN WALLS. Bloomfield Hills, Mich.: Cranbrook Institute of Science, Bull. 19, 1942: i–xiv, 1–785, 2 pls., figs. 1–197. $6.50.

AN active and accomplished researcher seldom takes time off to prepare a comprehensive treatise on his specialty. When he does he is apt to enter into great detail, to emphasize controversial points, and of course to think and write in so technical a manner, that his product is of use only to his fellow specialists (who will likely be in least need of such a resumé). Or he may underestimate his readers' capacities and delete technical information so largely that his book is not truly informative.

Walls set his course between these two extremes—a very difficult bit of navigation. Scientists will long for more documentation, for more thorough treatment of alternative theories, often for more detail; non-scientists will be repelled from time to time by technicalities, even though the job of sugar coating has been very skillful. The compromise in treatment and verbage has in general been so masterful, however, that the book will serve very well the needs of a wide range of readers. Oculists, medical practitioners, general zoologists, psychologists, and wideawake persons in many non-biological fields, now have available a full and understandable account of how all the vertebrates see, of what they see, and of how the seeing as they do fits them for the life they lead. The varying anatomy, function, phylogeny, and adaptation of the vertebrate eye are all dealt with in this large and handsome volume. Never before has so much light been shed at one time on an animal organ.

This is a tremendously interesting and valuable book. It deserves a place on thousands of library shelves.

Sex Hormones. Edited by F. C. KOCH AND PHILIP E. SMITH. Biological Symposia, Vol. IX. Lancaster, Pa.: The Jaques Cattell Press, 1942: i–x, 1–146, 46 figs. $2.50.

IN the ninth volume of BIOLOGICAL SYMPOSIA there are published the contributions to the Symposium on Sex

Hormones, which was one of the features of the Fiftieth Anniversary Celebration of the University of Chicago, and to the Symposium on Hormonal Factors in Sex Inversion, which was held at the April, 1942, meeting of the American Association of Anatomists.

The first series treats the actions and metabolism of the sex hormones. The foreword is by FRANK R. LILLIE, and the four papers are: "The Comparative Biology of Testicular and Ovarian Hormones," by CARL R. MOORE; "The Comparative Metabolic Influences of the Testicular and Ovarian Hormones," by A. T. KENYON; "The Metabolism of Estrogens," by EDWARD A. DOISY, and "The Excretion and Metabolism of Male Sex Hormones in Health and Disease," by F. C. KOCH. Together these contribute an important contribution in one of the most active branches of current biological progress.

The second series of symposia deal with "Hormonal Factors in the Inversion of Sex." The papers are by C. H. DANFORTH R. R. HUMPHREY, R. R. GREENE, and R. K. BURNS, JR., and deal with sex-inversion experiments, respectively on birds, amphibians, the rat, and the opossum. An understanding of the basic biology of sex is markedly advanced by these studies.

The Crayfishes of Florida. By HORTON H. HOBBS, JR. University of Florida Publication, Biol. Sci. Ser., 3, 1942: i–v, 1–179, pls. 1–24, maps 1–11, figs. a–c. $2.25 plus postage.

THE University of Florida has become an outstanding center of natural history research. Stimulated by their location in a region where the fauna is extremely rich and varied, yet very poorly known, the Florida zoologists are engaged in thorough studies of Southeastern animals. Working with rare enthusiasm and energy they are constantly making discoveries of great taxonomic, distributional, ecological and evolutionary significance. Among the most notable of these studies is that of Hobbs, on the crayfishes of Florida.

This student has found an amazingly rich cambarid

fauna in Florida—42 species and subspecies. In defining these forms Hobbs has been led to a revision of the classification of the Cambarinae. Instead of the single genus *Cambarus* he recognizes six genera, including the remarkable new blind genus, *Troglocambarus*.

Because Florida has so recently emerged from the sea, Hobbs has been able to trace plausible routes by which the several speciating lines may have entered the state. As a result of thorough field work, present distributions and habitats have been well defined and correlated. Isolation is indicated as an important speciational factor, particularly in the varied topography and separated stream systems of the Florida panhandle. Some of the characters are held to be of adaptive significance. Speciation has apparently been rapid as well as extensive, both in open waters and in caves. Though the author does not stress this point we seem to have here a splendid example of the theory that evolution is ordinarily a slow process, because it is retarded by the biotic saturation of old lands and waters. When this repressive force is weakened, speciation becomes free and rapid. When almost totally unoccupied habitats become available, as they did when the rift lakes of Africa were born, and when Florida rose from the sea, evolution may become explosive. Nature abhors a vacuum.

Studies on the Origin and Early Evolution of Paired Fins and Limbs. By WILLIAM K. GREGORY AND HENRY C. RAVEN. Ann. N. Y. Acad. Sci., 42, 1941: 273–360, figs. 1–34, pls. 1–5, $1.00.

THESE new attacks on old problems bear on some of the fundamental concepts of phylogeny. The authors counter a generally accepted tenet of phylogeny, that highly specialized groups are not the stuff out of which major phyletic lines are born. The ostracoderms—weird mailed fishes of the Paleozoic—are regarded as the ancestral stock of fish evolution; not as offshoots which were preserved by reason of their specialized exoskeleton, while contemporary and less specialized kinds, theoretically

more logical as ancestral stocks, died without leaving a known trace. The external armor is held by Gregory and Raven to have become reduced and modified in the other fish groups to form not only the bones of the skull but also the skeleton of the appendages. The views of these authors are dependent on certain assumed phyletic relationships of the major fish groups. But since these main lines sprang into existence almost simultaneously soon after the origin of the vertebrates, and since the record is highly incomplete, one may with at least equal plausibility assume other lineages, which would call for radically different concepts of comparative anatomy and evolution. The simple skeleton of the fold-like paired fins of the primitive Actinopteri, as exemplified by *Polyodon,* suggest what I still regard as the most plausible theory as to the mode of origin of the limbs, a theory that is compatible with the idea that generalized animals alone are capable of embarking on major evolutionary journeys.

The main portion of these studies deals with another problem, the transformation of the paired fin of the Pisces into the pentadactylate limbs of the Tetrapoda. Here the authors come out of the quagmires (as the ancestors of the tetrapods did) to tread on firmer ground; to display more convincingly their mastery of paleontology, anatomy and taxonomy; and to contribute more securely to our ideas on the evolution of the vertebrates.

ARTICLES ON SPECIATION AND EVOLUTION

Adaptive Modifications for Tree-trunk Foraging in Birds. By FRANK RICHARDSON. Univ. Calif. Publ. Zool., 46, 1942: 317–368, pls. 23–24, figs. 1–16. $0.75.—Many parallel and some compensatory adaptations are demonstrated to fit certain birds for the tree-trunk foraging by which they obtain their food. The adaptations are regarded as parallel instead of convergent, because they involve homologous rather than nonhomologous structures. This criterion, also advocated by Abel, is preferred by the author over the more common one that is based on degree of relationship. There are obvious advantages in each point of view, and the issue is confused by applying the same terms to

distinct concepts. I would rather contrast homologous and non-homologous adaptations, as well as distinguishing between parallel and convergent ones. I agree with Richardson's view that "natural selection seems to be confirmed by the very existence of structural adaptations," but regard as indefensible his restriction of the adaptation concept to "structural modifications mechanically suited to the use to which they are put." To delete habits from such concepts as adaptation and homology is to revert to the age when morphology was unduly emphasized, and hinders clear evolutionary thinking.

The Osteology and Myology of the California River Otter. By Edna M. Fisher. Stanford University: Stanford University Press, 1942: i–vi, 1–66, figs. 1–37 (offset printing). $1.50.—This study was undertaken as an accessory to the author's researches on the aquatic adaptations of the sea otter. The river otter shows a lesser degree of modification for life in water. In order to permit the desired comparisons, the anatomy of the river otter had to be worked out in fine detail.

Geographic Variation in Garter Snakes of the Species Thamnophis sirtalis in the Pacific Coast Region of North America. By Henry S. Fitch. Am. Midland Nat., 26, 1941: 570–592, figs. 1–3.—This report is a sequel to the same author's outstanding study of speciation in *Thamnophis ordinoides*, which was reviewed in these columns (Am. Nat., 75, 1941: 384–386). The species *sirtalis* proves to be the more uniform in habitat preference and in characters. It shows some geographical variation in the ventral and caudal scutes, but the systematic significance of these variations is discounted. Regional variations in coloration, which form a different and a more consistent geographic pattern, are used to diagnose four western subspecies. There are marked local variations within these subspecies, and sharp distinctions occur on the two sides of barriers. The local variations in *sirtalis* often follow those in *ordinoides*. In both species the adult size and the number of scutes decreases toward the north, toward higher elevations and toward the coast. There are parallelisms too in coloration, and some of the local races of the two species that live together are remarkably alike. The several color types appear to be concealing in their local environments, and are therefore held to be of probable adaptive significance.

Some Aspects of Evolutionary Theory. By GEORGE M. ROB-
ERTSON. Fort Hays Kansas State College Studies, Gen. Ser., 4
(Sci. Ser. 1), 1942: 113-167.—The taxonomy and phylogeny of
the lower vertebrates is the primary subject of these essays. There
is offered an outline classification of the Chordata, reflecting the
discoveries and opinions of paleontologists and embodying some
original ideas. Consideration is also given to the bearing of cer-
tain paleontological and embryological data on some problems of
homology. Principles of phylogeny are discussed and the con-
clusion reached that phylogeny can become a science only with the
development of tested generalizations.

Statistical Genetics and Evolution. By SEWALL WRIGHT.
Bull. Am. Math. Soc. 48, 1942: 223-46, figs. 1-10.—A treatment
of the statistical consequences of the evolution theory, with
particular emphasis on changing gene frequencies.

SHORTER ARTICLES AND DISCUSSION
FURTHER NOTES ON DIFFERENTIAL SELECTION OF VARIANT JUVENILE SNAKES

A comparison of juvenile and adult populations of the smooth green snake, *Opheodrys vernalis* (Harlan), supports the con-clusions of E. R. Dunn (1942) as to differential selection in juvenile snakes (Inger, 1942). A similar study of *Thamnophis radix* Baird and Girard, using 157 adults and 160 juveniles, affords further information on this subject. The specimens examined are from the adjacent Cook, Lake, and McHenry Coun-ties, Illinois, and Walworth County, Wisconsin, and are contained in the collections of the Field Museum of Natural History. The juvenile specimens are 200 mm or less in length; of the adults none is smaller than 400 mm, which is approximately the size of the smallest reproductively mature individual (Cieslak, 1938). I wish to express my gratitude to Messrs. Karl P. Schmidt, and Clifford H. Pope, and Dr. Carl L. Hubbs for their valued criticism and aid and to the Field Museum of Natural History for the use of its laboratories and collections. I am also indebted to Dr. Sewall Wright, of the University of Chicago, for his comments on the data contained in this paper.

A feature of the comparison between the two age groups shown in Table 1 lies in the difference of the means of certain scutellation characters. The difference in the means between the juvenile and adult series is significant in the ventrals and caudals only. It is interesting to note that there is uniformity in the differences which are significant.

The juveniles show a larger coefficient of variability than the adults in every character examined, indicating a greater variabil-ity of the juvenile population, from which a differential selection is inferred. In the case of the ventrals, the reduction of the coefficient of variability is not brought about through the loss of individuals from both extremes of the observed range of the char-acter, as would be expected. The reduction is due instead pri-marily to the elimination of individuals from the lower extreme. The same trend is apparent in the caudals. This is illustrated in Figures 1 and 2.

The explanation of such differential selection is not clear. The negative selection of anomalies may well be due to their associa-

TABLE 1

COMPARISON OF SCUTELLATION OF JUVENILES AND ADULTS OF
THAMNOPHIS RADIX

Character	Sex	Age	Mean	Difference of means	Coefficient of variability	Number examined[1]
Ventrals	♂	adult	156.71	2.15 ± .545	2.02 per cent.	85
	♂	juvenile	154.56		2.35 " "	74
	♀	adult	151.05	2.18 ± .565	2.13 " "	72
	♀	juvenile	148.87		2.61 " "	86
Caudals	♂	adult	73.75	1.78 ± .603	4.70 " "	65
	♂	juvenile	71.97		4.95 " "	71
	♀	adult	64.80	2.42 ± .540	4.35 " "	56
	♀	juvenile	62.38		6.36 " "	84
Infralabials	♂ + ♀	adult	9.80	.02 ± .03	4.30 " "	308
	♂ + ♀	juvenile	9.78		5.25 " "	318
Supralabials	♂ + ♀	adult	7.19	......	5.80 " "	313
	♂ + ♀	juvenile	7.19		6.56 " "	318
Postoculars	♂ + ♀	adult	2.98	......	4.62 " "	314
	♂ + ♀	juvenile	2.98		4.95 " "	318
Temporals (2nd row)	♂ + ♀	adult	2.18	.02 ± .03	19.4 " "	312
	♂ + ♀	juvenile	2.16		20.7 " "	320

[1] Number refers to specimens in ventrals and caudals. Number refers to sides of heads in other characters.

tion with other, less evident, physiological deficiencies. By way of further speculation, it may be pointed out that the evolution of snakes appears to exhibit a trend toward an increase in flexibility—a flexibility necessarily accompanied by an increase in the strength of the spinal column. To an animal without appendages, flexibility of body is obviously of great survival value. Increase in strength of the vertebral column (over that of lizards)
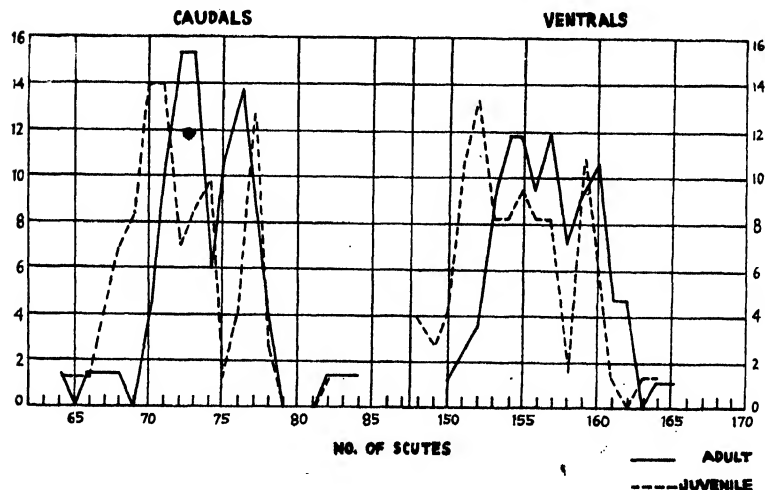
FIG. 1. Frequency distribution (percentage) of abdominal scutes of males of *Thamnophis radix*.

is produced by additional articulations between the vertebrae. These additional zygapophyses, the zygosphenes and zygantra, have added so much to the rigidity of the articulation between vertebrae that any bend in a snake's body usually involves at least ten vertebrae. A net increase in flexibility can come about therefore only by adding to the number of vertebrae.
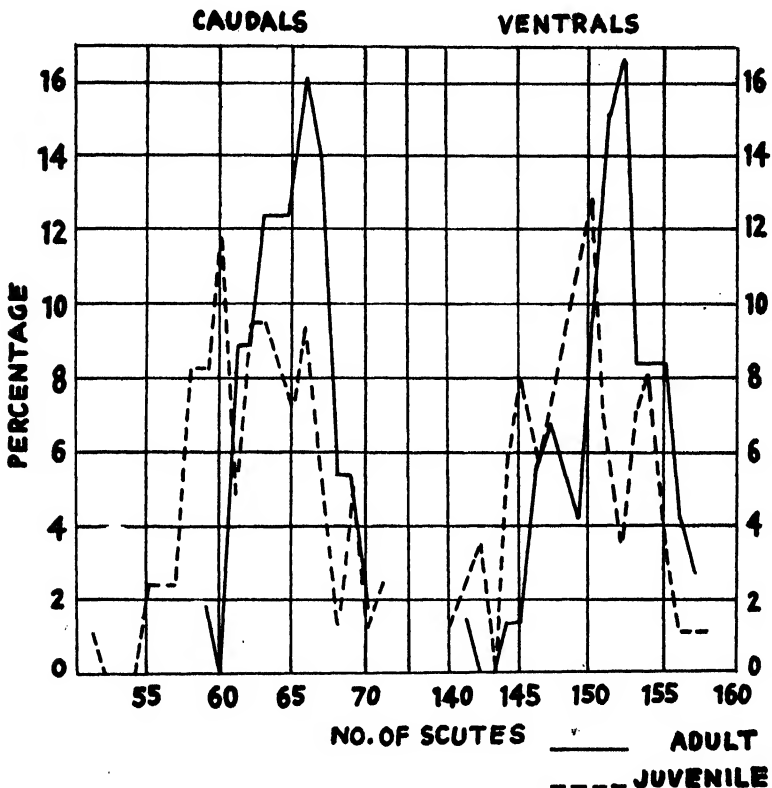
FIG. 2. Frequency distribution (percentage) of abdominal scutes of females of *Thamnophis radix*.

The number of ventrals (and caudals) is a measure of the number of vertebrae. A reduction in the number of vertebrae (and the correlated reduction in ventrals) may be thought to have negative survival value because it would decrease flexibility, and natural selection would thus tend to eliminate individuals showing such a reduction. That this is true may be seen from the data in Figs. 1 and 2.

Other factors must be supposed to limit the maximum of vertebrae. The difference between the mean of the ventrals (and

caudals) of the juveniles and the mean in the adults obviously does not indicate a continuing increase in the number of ventrals in this species; for *Thamnophis radix* with a mean of ventrals of 154 may be supposed to have maintained about this number for several millennia.   If a difference as large as shown, existing between two generations, were continued from generation to generation, this species would have upward of 1,000 ventrals in the space of two centuries.   The change in the mean number of ventrals (and caudals) from juvenile to adult therefore does not represent an evolutionary advance or even a genetic change, but a selection mechanism for maintaining the number of ventrals at the norm established in the evolution of the species.

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A BASIS FOR OSTENSIBLE REVERSAL OF EVOLUTION

THE non-reversibility of evolution is a biological law which is indisputably valid as a generality, yet occasionally there appears a phylogenetic sequence which seems to result from a reversion to an ancestral condition.   An example, to mention but one, is the occurrence of teeth in the lower jaw of the amphibian genus *Amphignathodon*.   This condition is not found in any of the other genera of the Hylidae, a relatively advanced family, nor in any other anuran.   In all the phylogeny of the anura, no teeth are known until we reach this specialized family.   Dr. Norman Hartweg has suggested that such exceptions may be special cases of the law of non-reversibility rather than contradictions, inasmuch as these reversions do not necessarily entail a re-evolution of structures completely lost.   We can think of a reversion as a phyletic atavism—the evolutionary unmasking of long-hidden primitive characteristics.   Conceivably all the genetic and developmental requirements for an atavistic character might be pres-

ent, but suppressed in a specialized organism. There is good experimental evidence for such a hypothesis.

SUPPRESSOR GENES

If we conceive of an apparent reversal of evolution as due to a reappearance of suppressed genetic potencies, we must first seek known cases of the suppression of one gene by another. This is not the same as the dominant-recessive relationship between alleles, but is suppression of a gene in one locus by a gene in another. The term epistasis is applied when one gene has an effect of its own and also suppresses another which is not its allele. Perusal of any genetics text will provide numerous examples. For instance, in *Drosophila,* the factor *su* in chromosomes III will suppress purple eye color, for which the genes are in chromosomes II (Bridges, 1932). Thus the genotype *pr pr* II with + + III produces purple eye, as the normal wild-type (+) genes in chromosome III do not interfere. Introduce the suppressor, *pr pr* II with *su su* III, and wild-type eyes result. By such a mechanism, a race of purple-eyed *Drosophila* might revert to wild-type without any change in the purple factor itself, merely as a result of the introduction of the suppressor. Of a somewhat different type is the suppression of sepia eye by the white eye factor. A fly homozygous for both is white-eyed. Replacement of one *w* gene by w^+ allows the sepia to show. Epistasis and suppression occur in all types of organisms. The albino factor in mice hides any genetic color pattern present, and gray prevents the expression of black. In fowls there is a color suppressor, and another in squashes. The influence may be partial or qualitative, in which case the gene is known as a modifier, and a whole series of modifiers may be present, producing a graded series. Mice homozygous for spotted coat may vary from almost solid color to almost complete lack of color, depending upon the number of modifiers present. The sepia mentioned above and certain other eye color factors can be considered modifiers of the wild allele of white.

An instance which might easily be termed reversal of evolution by one not sworn to uphold the paleontological postulates is polydactyly in the guinea pig (Wright, 1935). Selection produced heterozygous five-fingered, four-toed guinea pigs from the four-fingered, three-toed wild type. This is not a case of a single-factor suppressor, but it is genetically controlled atavism in that favorite of the paleontologist—digit pattern.

Clearly there is an abundance of genetic evidence favoring the possibility that a gene may be carried through a number of generations without affecting the phenotype, or having only a partial effect, and subsequently recover its full phenotypic expression. How long such a suppressed condition might persist, having no selective value, may presumably be judged from the persistence of vestigial organs which are neither helpful nor harmful.

Evidence from Experimental Embryology

It might be argued that in taking evidence from the action of single genes, we are over-simplifying the problem. A taxonomically important modification involves a whole organism, not a single-factor character. It must be kept in mind, however, that ontogeny is a synergy of catenated phenomena, and failure of a single process can alter all subsequent events. The giant-lethal mutation in *Drosophila* is a single-gene effect inhibiting the pupation hormone (Hadorn, 1937). As a result, the larva continues to grow and never pupates. Injection of the hormone allows the whole complicated process of pupation to continue. Action of a single gene alters the entire life of the individual. Experimental embryology has revealed dependent sequences to be the rule. As an example could be taken the gill, which depends upon the presence of ectoderm over the mesoderm gill field. This ectoderm normally forms gill, but if removed from the influence of the gill field before its fate is determined, it does not form gill. Reciprocally, flank ectoderm will form gill if transplanted to the gill field. Without altering the genetic constitution of that ectoderm, we can call forth or suppress its gill-forming potencies. Similarly, the gill field is determined by preceding organizers, and so on, back to the primary organizer. It is not too much to postulate that somewhere in this chain of events a single gene could act to prevent formation of gills.

A temporary suppression of gills occurs in the *Desmognathus fuscus* larva while it remains on land (*cf.* Noble, 1931). In *Breviceps* and some other microhylid toads, gills are entirely lacking. Here experimental embryology opens the theory to experimental attack. If it is the ectoderm in *Breviceps* that lacks gill-forming potencies, transplantation to the gill region of a gilled amphibian should reveal that lack. Reciprocal transplants would determine whether the inhibition acted upon the mesoderm gill field, or upon the ectoderm, or both. Other larval organs are open to the same approach.

LIFE CYCLES REVEAL GENE SUPPRESSION

We can not study the physiology of gene suppression in an evolutionary series, but some life cycles reveal a comparable phenomenon within a shorter span of time. One such is the parthenogenetic-bisexual cycle that occurs in some insects, rotifers and cladocerans (Shull, 1925). A wingless female aphid, coming from several generations of parthenogenetic females, will give birth parthenogenetically to a female that develops wings. The chromosome numbers are the same and there has been no opportunity for the introduction of new genic material, so we can only conclude that the wing-producing functions were suppressed. Moreover, a series of parthenogenetic females will end with females which produce males or females capable of sexual reproduction. Clearly whole complexes of genetic potentialities can survive a number of generations without phenotypic expression, to reappear at a later time, all within a normal life cycle.

CONCLUSION

There are already known to exist all the processes which would be requisite for evolutionary atavism. Genic behavior and embryological process supply the necessary mechanisms, and variable life cycles show that such masking and unmasking occur naturally. In the fossil record, such release of masked characteristics would have the appearance of a reversal of evolution, whereas it actually would be a re-emergence of unaltered ancestral characteristics. It would not be reversal in that the structures and functions were not evolved anew. With regard to the interpretation of phylogeny, however, it would serve essentially the same as a reversal.

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EFFECTS OF SILVER NITRATE ON THE PIGMEN-
TATION OF DROSOPHILA

RAPOPORT (1939) has published preliminary data on the production of phenotypic modifications in *Drosophila melanogaster,* by raising the larvae on food containing sublethal doses of certain chemical compounds. Some of these modifications appeared to resemble certain mutant types known in the same species; these modifications are considered phenocopies. In particular, treatments with silver lactate and some other (not specifically named) silver compounds produce, according to Rapoport, "sharp reduction of the body color, perfectly resembling extreme alleles of the mutant yellow." The observations described below confirm and extend those of Rapoport on the effects of treatments with silver salts.

The concentration of silver nitrate which is sublethal for Drosophila larvae has been determined. Stender jars with an inside diameter of 2 inches were provided with 10 cc of the regular cornmeal-molasses-agar food to which definite amounts of a 10 per cent. solution of silver nitrate had been added during the preparation. Five series of jars were prepared containing 0.1, 0.05, 0.01, 0.005 and 0.001 grams of silver nitrate per 10 cc of food, respectively. Flies were allowed to oviposit on spoons with the regular silver-free food. Known numbers of freshly hatched larvae, no more than two hours old, were transferred to the experimental jars and allowed to develop to the adult stage. The jars with the silver-treated food were, as far as possible, protected from light. In the jars with 0.1 and 0.05 grams of the silver salt all the larvae died. The concentration of 0.01 grams of silver nitrate per 10 cc of food was found to permit enough larvae to survive. This concentration was used as the standard in all subsequent experiments.

The wild type of *Drosophila melanogaster* raised in jars with the standard concentration of $AgNO_3$, as well as in jars with 0.005 grams per 10 cc, showed a very pale body color. The flies hatched in the jars with 0.001 grams of $AgNO_3$ per 10 cc were apparently normal. The body color in the modified flies is a pale yellowish gray, distinctly different, contrary to the statement of Rapoport, from that in any allele of yellow with which the writer is familiar. It resembles most closely that in the sex-linked recessive mutant straw, but is even somewhat paler and grayer than straw. Not only the integument proper but also the

bristles and the microchaetae on the fly's body are paler than normal. The dark bands on the abdominal segments in both sexes are still visible but are much less distinct than normal. The chitin is delicate and gives the impression of being softer or more elastic than normal. If the silver-treated flies are a phenocopy of any known mutant, this mutant is straw and certainly not yellow or "silver."

Aside from the wild type, certain mutants of *Drosophila melanogaster* were also tried on the standard silver-containing food. The ebony mutant becomes strikingly paler, but by no means as pale as the treated wild type. The treated ebony is about intermediate in coloration between the untreated wild-type and the untreated mutant sooty, which is a lighter allele of ebony; the fly is, however, suffused gray, showing no distinct trident pattern on the thorax and no distinct dark bands on the abdomen. The treated black-speck becomes lighter than the untreated speck without black. The mutant yellow raised on the standard silver-containing food is paler than the untreated controls, but the difference is by no means as striking as that between the treated and untreated wild-type or ebony flies. Finally, the mutant straw changes little if at all as a result of silver treatment. Two other species, which are normally much darker in the body color than *D. melanogaster*, namely *D. pseudoobscura* and *D. virilis*, have been tried. When raised on the standard silver-containing medium they become very much paler than the untreated controls, but they preserve a smoky brownish gray coloration which is much darker than that in the straw mutant of *D. melanogaster*.

Aside from the changes in pigmentation of the integument, all the treated flies of the three species examined show also changes in the color of the pericardial cells and of the Malpighian vessels. The pericardial cells in the untreated flies contain little or no pigment; in the treated ones these cells are of a dark brownish red color. Two rows of these cells are clearly visible through the body wall of the dorsal surface in the treated flies. It may be desirable to point out specifically that the red pericardial cells appear also in treated straw flies in which little change of the body coloration is perceptible. The red coloration of the pericardial cells is clearly present not only in the adult flies but also in the larvae developing on the silver-containing food. The normal greenish pigment of the cells in the Malpighian vessels is changed to a reddish brown, in the adults as well as in the larvae.

An attempt has been made to determine whether or not there is a period in the larval development during which the silver treatment produces its effects on the coloration of the adult fly. Freshly hatched wild-type larvae of *D. melanogaster* were collected at one-hour intervals and placed in jars with silver-free food. From time to time a group of larvae of known age were transferred to the standard silver-containing food, allowed to remain there for 24 hours, and returned back to the normal food to complete their development. The larvae which have been kept on silver-containing food from hatching from the eggs to the age of 24 hours gave rise to adults with a straw body color, pinkish pericardial cells and greenish Malpighian vessels. Larvae treated at any age older than 24 hours give straw-colored adults with deep orange pericardial cells and brownish Malpighian vessels. There is, thus, no pronounced sensitive period during which the effects of the silver treatment would be produced.

The physiological mechanisms intervening between the ingestion of the silver salt by the larvae and the appearance of the abnormal colorations in the adult are unclear. It is, however, doubtful whether the silver-treated flies may be justly called phenocopies of straw or of any other known mutant. Indeed, aside from a slight difference in the coloration of the integument, the straw flies do not show the changes in the pericardial cells and in the Malpighian vessels which are so characteristic of the silver-treated flies.

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ECOLOGY, EVOLUTION AND SOCIETY[1]

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ECOLOGY deals with the interrelationships between the organism and its environment, both physical and biotic. The concept of the organism, however, is complex and various levels of biological organization occur. Viruses, cells, multicellular organisms, metameric organisms, metamorphic organisms, colonial organisms, species populations, aggregated populations, cyclomorphic populations, sex pairs, family units, social units and certain types of ecological communities, each represent a level of individuality with organismic attributes.[2,3] Each living unit, in other words, has its external environment.

The environment of a cell, if it be a protozoan, may be treated by the ecologist, but the environment of a cell, if it be in a tissue or organ of a multicellular organism, is usually treated by the physiologist. The environment of a red blood cell is thus studied by a physiologist, but the environment of a malarial parasite within the erythrocyte is the province of the parasitologist who comes within the broad field of ecology. The environment of an embryo is studied by the embryologist while the environment of a larva is within the province of the ecologist. The social environment of an individual human is studied by the sociologist, the psychologist, the economist, the

[1] Presidential address delivered before The Ecological Society of America, Dallas, Texas, on December 29, 1941.

[2] A. E. Emerson, *Amer. Midland Nat.*, 21: 182–209, 1939.

[3] F. E. Clements and V. E. Shelford, *Bio-Ecology*. vi + 425 pp. New York: John Wiley and Sons, 1939.

anthropologist, the geographer and the historian, while the social environment of the individual ant is again the interest of the ecologist who also treats the external environment of the ant colony. If the environmental factors are correlated, as they often are, with hereditary, physiological, developmental or behavioristic phenomena, they are immediately brought into the reports of the geneticists, physiologists, embryologists and psychologists.

I should not interpret the difficulties involved in setting arbitrary lines of demarcation between ecology and various sciences as indicative of a lack of a central ecological theme. Granting the existence of various levels of integration among organisms and the complexity of the environmental effects, a basic dichotomy is still possible between the living unit and its environment even without precluding greater units that might incorporate both organism and environment.

I should rather interpret the overlap of concepts and interests as indicative of the great benefit that ecologists may derive from an understanding of the principles discovered by other sciences and the great value that ecology can be to other scientific fields.

Ecology has made some of its greatest advances in the study of the integrative patterns of organization in the ecological community and the dynamic effects of environmental factors upon the organism. These may be considered as pertaining to the three dimensions of space. A fourth dimension involving time is the base of reference in the study of ecological succession which may be likened to the ontogenetic sequence of the individual organism. Migration together with diurnation, aspection and annuation are also phases of these space-time dimensions.[4] An extension of the spatial and time dimensions is found in the different ecological origin of various species in the community succession. In the Indiana dunes bordering Lake Michigan, a species of termite (*Reticulitermes tibialis*) is found from the foredunes

[4] *Ibid.*

through the oak stages, but another species (*Reticulitermes flavipes*) is found in the climax beech-maple forest on both sand and clay. The first species, however, is found in the relatively dry post-climax and pro-climax valley woodlands of the states west of the Mississippi and the second species is found in the relatively mesic seral stages in the eastern states. These species also have diurnal and seasonal cycles. An understanding of the time sequence of communities in one area thus involves a knowledge of the spatial origins and various ecological adjustments of the species as well as their immediate community relations.

I propose in the limited time at my disposal, not to mention my limited capacity and perspective, to place emphasis upon a fifth dimension of ecological study, also involving time, namely evolution. We are all aware in our ecological studies that the functional correlation between an organism and a factor in the ecological community is not to be understood wholly by studying the present relationship. Even a succession of communities is not completely explained through an understanding of present day factors operating on existing species. The dynamics of an existing interaction has a chronological background that stretches further than the chronological succession of existing factor patterns. A species of tiger beetle on sand in the early stages of a dunes succession attained its bilaterality before its segmentation, its compound eyes before its tracheae, its legs before its wings, its mandibles before its larval stage and its predatory adjustments before its adjustments to narrow ranges of soil moisture. Not only this, but most of the adaptations to this present environment which help to explain why this species is found where it is and why it is absent from other somewhat similar habitats were developed in part at least in other ecological situations inhabited by the ancestors of the existing species (an aspect of preadaptation). In addition, it retains in its organization and in its development, functionless characteristics which were

functional in its ancestors living under different ecological conditions. It is as impossible for an ecologist to explain certain obvious facts of ecological pattern by recourse only to the existing spatial dimensions and the time dimension of the successional sequence, as it is for an embryologist to explain certain obvious facts of embryological pattern through an investigation limited to the dynamics of the immediate organic spatial pattern in its ontogenetic sequence.

I may sum up this introduction by stating that an understanding of evolution is important to an adequate understanding of an organism, an organism in its environment, or an ecological community. Ecologists may be proud of the fact that they are lineal scientific descendants of the nineteenth century naturalists who contributed to our knowledge of the mechanisms of evolution. Ecology, to use one of Wheeler's similes, is hardly more than a branch from the natural history stolon which is so slightly differentiated that it may be a question whether it is a branch or the stolon itself.

I am reminded of the discussion between W. C. Allee and the ornithologist, Rudyerd Boulton. Allee stated that ecology was scientific natural history that became self-conscious at the turn of the century. A light dawned on Boulton who remarked, "Now I know what an ecologist is; an ecologist is a self-conscious naturalist." I hardly know of a better definition and we may well pride ourselves in our capacity as naturalists along with the taxonomists and geneticists who would also claim close scientific kinship to Lamarck, Darwin, Wallace, Haeckel and Weismann. However, we must carry on the inquiries of these men if we are to point to them with pride. What can modern ecology contribute to an analysis of evolutionary dynamics?

I think we may well leave the causes of hereditary variation to the geneticists. It is true that environmental factors such as chemicals, heat, ultraviolet light and radio-active emanations may induce mutations and

there may even be some ecological distribution of such causative factors. Chance chromosomal and gene mutations may immediately effect the ecological adjustments, sometimes in a beneficial manner (another aspect of preadaptation).[5] However, mutations, especially in their complex patterns, seem to have no ecologically directed functional relation to the environment, leaving selection and isolation aside.

Since the days of Lamarck, many investigators have sought for data on the inheritance of acquired, environmentally induced, somatic characters. Although it is probably too early to become rigidly dogmatic on this point, I do believe it is safe to say that the data in support of any such Lamarckian mechanism are largely lacking and that other mechanisms seem to be far more adequate in explaining the vast majority of known evolutionary phenomena.

The ecologist has much to contribute to two main phases of evolutionary dynamics, however, namely isolation and natural selection. In other words, ecological factors have an important influence on evolutionary divergence and adaptation. Also the initial stages of an evolutionary divergence involve population units. Species, subspecies and races are population concepts and the ecologist is interested in the intraspecific environment of the individuals within such populations and the environmental effects upon population physiology and integration.

All evolutionary divergence involves reproductive isolation in some form. As far as we know, genetic variation within populations is constantly occurring although the rate may differ in different genes, in different chromosomes, in different organisms and in different populations. Not only do qualitative genetic differences occur in isolated populations which can not spread their chromosome patterns to their closest genetic populations without interbreeding, but a quantitative shift in allele

distribution may distinguish recently isolated populations. If all other factors remained constant except that a different sized population became isolated from the original population, a gradual divergence would occur through accidental fluctuation of the concentration of single genes.[6,7] Large fluctuations in the effective breeding population (population waves) act as partial isolating agents. The elementary evolutionary process is often a change of gene-frequency rather than mutation.[6] Also larger populations are likely to show wider ranges of variability than relatively smaller populations because of the tendency of closely inbreeding populations to develop homozygosity.

There are numerous types of isolating mechanisms which prevent population interbreeding. Lack of sexual reproduction in parthenogenetic and vegetatively reproducing organisms obviously prevents the sharing of genetic systems except through clonal descent.[8] In sexual species, fertility impairment, inviability of the hybrids and adult sterility all contribute to reproductive isolation, but are essentially physiological, genetic or embryological phenomena. Isolation of populations through geological time may produce chronological clines, but these are primarily the problem of the paleontologist. The ecologist, however, may make important contributions to the concepts of geographical, habitat, annual, seasonal, diurnal and sexual isolation.

Geographical isolation (which could be more exactly subdivided into spatial and topographical isolation) is generally accepted as having an important bearing upon evolutionary divergence. In order to analyze the complex factors correlated with geography, however, it is necessary for the ecologist to search for cases where one

[6] S. Wright, "The Statistical Consequences of Mendelian Heredity in Relation to Speciation." pp. 161–183. In J. Huxley, "The New Systematics." Oxford, 1940.

[7] N. W. Timofeeff-Ressovsky, "Mutations and Geographical Variation." pp. 73–136. In J. Huxley, "The New Systematics." Oxford, 1940.

[8] K. B. Raper and C. Thom, *Am. Jour. Bot.*, 28: 69–78, 1941.

or a few factors differ while others remain constant. Such naturally occurring cases sometimes have all the analytic values of controlled experiments. For example, populations of Amazonian ant-thrushes differ on the right and left banks of the Amazon, although there would seem to be no correlated habitat differences.[9] These birds, although capable of flight, seem to be confined to deep woods and avoid crossing such open spaces as wide rivers. The only known correlated factor with such evolutionary divergence, therefore, is the isolation of the populations by means of the river barrier. Closely similar ecological habitats often are inhabited by different closely related species in such situations as the opposite banks of the Grand Canyon, separated mountain ranges, oceanic islands, separate caves within larger cave systems, etc. When such topographical localities differ ecologically, differential selection is probably also operating in addition to isolation, thus complicating the factoral analysis.

Habitat isolation (sometimes called ecological isolation[10]) of species in the same geographical area is a common enough observation. Worthington[11] gives interesting cases of speciation of fishes (*Haplochromis*) in Lake Victoria correlated with different types of food. Fulton[12] experimentally crossed two subspecies of crickets (*Nemobius fasciatus fasciatus* and *N. fasciatus tinnulus*) with song distinctions living in the same geographical region but in different habitats. Successional series often show a sequence of closely related species which are geographically contiguous in at least portions of their ranges. Probably the best examples of habitat isolation, however, are to be found among the monoxenous herbi-

[9] R. Boulton, personal communication.

[10] T. Dobzhansky, ''Genetics and the Origin of Species.'' p. 257. (2d edition.) New York: Columbia Univ. Press, 1941.

[11] E. B. Worthington, ''Geographical Differentiation in Fresh Waters with Special Reference to Fish.'' pp. 287–302. In J. Huxley, ''The New Systematics.'' Oxford, 1940.

[12] B. B. Fulton, *Ann. Ent. Soc. Amer.*, 26: 368–376, 1933.

vores,[13] parasites, and symbiotic guests of social insects.[14,15] Of course such cases usually involve adaptation. In some instances, however, natural selection may be a minor factor. Certain herbivores may be forced to eat plants which they do not normally eat and adults raised from such experimental stocks will oviposit on the new plant when given a choice (Hopkin's host-selection principle). Thorpe[13] cites an experimental case of host selection by an Ichneumonid (*Nemeritis canescens*) and concludes, "The theoretical importance of such a conditioning effect is that it will tend to split a population into groups attached to particular hosts or particular food-plants, and thus will of itself tend to prevent cross-breeding." Territoriality among breeding birds also is the result of conditioning and the young may return to the locality of their origin for subsequent breeding. Thus an isolating mechanism between populations in local regions may be inaugurated without ecological adaptation. Such territoriality might give rise to species divergence if similar ecological areas are sufficiently isolated. I cite an instance to illustrate the hypothetical action of such factors in the case of the Eastern and Western Wood Pewees (*Myiochames virens* and *M. richardsonii*) which overlap in wintering range in Central and South America but breed in separate areas without known adaptive adjustments. The species are remarkably close in their morphological and color characters, but differ somewhat in their calls.

Annual isolation within the same geographical and habitat areas may possibly have some effect upon the divergence of species with life cycles extending two years or more. The races of the periodical cicada (*Magicicada septendecim*) may be partially isolated in this way and the races of the Pink Salmon (*Oncorhynchus gorbuscha*)

13 W. H. Thorpe, "Ecology and the Future of Systematics." pp. 341–364. In J. Huxley, "The New Systematics." Oxford, 1940.

14 A. E. Emerson, *Ann. Ent. Soc. Amer.*, 28: 369–395, 1935.

15 C. H. Seevers, *Ann. Ent. Soc. Amer.*, 31: 422–441, 1938.

which breed in the same streams in alternate years are differentiated.[16]

Seasonal isolation is difficult to separate from possible selective forces, although statistical correlations may indicate that isolation rather than selection is sometimes the main cause of evolutionary divergence. Dobzhansky[17] has recently brought together several cases of seasonal breeding differentiation in otherwise closely related species occupying the same geographical and ecological habitats in at least part of their ranges. Differences in the flowering season of otherwise closely related species of plants and in the mating period of closely related species of butterflies are discussed. Piersol[18] gives a case of seasonal differentiation of breeding among salamanders (*Ambystoma jeffersonianum* and *A. maculatum*) in the same pond. However, seasonal isolation will be best illustrated by more data from experiments which prove no hybrid sterility or inviability. Hogben[19] refers to the case of two species of moths, one (*Eupithecia innotata*) feeding on *Artemisia* and the other (*E. unedonata*) emerging earlier and feeding on *Arbutus*. Pupae of the *Arbutus* species were cooled delaying their emergence and thus they were mated with the *Artemisia* species and fertile hybrids were produced.

Diurnal isolation may play a role in speciation. Larval microfilariae (*Wüchereria bancrofti*) usually show nocturnal periodicity in the human blood stream and are thus adapted for transmission by the night-biting mosquitoes (*Culex fatigans*, etc.). A non-periodical biological race or species of this parasite, morphologically similar to the periodical form, is transmitted by a day-biting mosquito (*Aëdes variegatus*) whose distribution is closely correlated with the non-periodic filaria in certain islands of

[16] F. A. Davidson, personal communication.

[17] T. Dobzhansky, ''Genetics and the Origin of Species.'' (2d edit.) New York: Columbia Univ. Press, 1941.

[18] W. H. Piersol, *Trans. Roy. Canad. Inst.*, 17: 57–74, 1929.

[19] L. Hogben, ''Problems of the Origins of Species.'' pp. 269–286. In J. Huxley, ''The New Systematics.'' Oxford, 1940.

the Pacific (Fiji, Samoa, Tokelau, Wallis, Ellice Islands, Philippines, and Tahiti).[20] This case illustrates the possible isolating effect of daily ecological fluctuations although the influence of selection is not eliminated.

Closely related species of fishes (*Pomoxis nigro-maculatus* and *P. annularis*), with similar food, food-habits and general behavior, occur together in the Illinois and Ohio rivers and a few interspecific hybrids are known. One (*P. nigro-maculatus*) shows a nocturnal activity rhythm and the other (*P. annularis*) is diurnal, thus indicating that these species are reproductively isolated through their different activity cycles. In the same family (Centrarchidae), the forms that hybridize freely have activity periods which are the same or broadly overlap.[21]

Sexual isolation involving both mechanical and psychological mating actions properly are ecological because one sex is a part of the environment of the opposite sex. Anything that interferes with the function of species or sex recognition may be an isolating mechanism. Crampton[22] states that sinistral and dextral snails (*Partula*) might be unable to mate thus mechanically isolating population units. Psychological or behavior isolation is particularly well shown in certain cases. Dice[23] has recently reported an overlap of the ranges of two species of mice (*Peromyscus leucopus* and *P. gossypinus*). In nature, hybrids are rarely found, but in the laboratory the two species interbreed readily and produce viable offspring. The Eastern and Western Meadow Larks (*Sturnella magna magna* and *S. neglecta*) do not seem to hybridize in nature but they do cross in the laboratory and produce offspring.[24] In the prairie habitats west of Chicago these two species may be found nesting in the same field.

[20] P. H. Manson-Bahr, ''Manson's Tropical Diseases.'' pp. 750, 950. (11th edit.) Williams and Wilkins Co., Baltimore, Md., 1940.

[21] O. Park, personal communication; data from C. L. Hubbs, D. H. Thompson, G. W. Bennett, C. L. Turner and W. P. Spencer.

[22] H. E. Crampton, *Carneg. Inst. of Washington, Publ.,* 410: 1–335. p. 188, 1932.

[23] L. R. Dice, *Jour. Mammalogy,* 21: 14–23, 1940.

[24] G. B. Saunders, personal communication.

Where possible, I have used examples of ecologically isolated species showing hybridization in order to analyze the factors with greater accuracy. The analytical value of subspecies and races in this connection is obvious. However, sterility between species is common even though ecological isolation is involved. Wright[25] states, "It appears probable that the more or less complete cross-sterility that permanently separates most good species from their nearest allies is usually a by-product of the gradual accumulation of genetic differences in populations isolated at first merely by geography, habitat, etc."

Darwin emphasized natural selection as the basic mechanism of evolution. To-day we feel that our knowledge of the genetics of variation and the role of isolation gives us a clearer picture of evolutionary dynamics. However, natural selection is still of tremendous importance, not so much as the prime factor in the origin of all species as it is in the explanation of practically all complex adaptation. Adaptive characters which have been analyzed genetically almost always are multiple gene effects and there is every reason for assuming that such complex gene patterns did not arise simultaneously. Adaptation is such a universal phenomenon and selection is such a satisfactory mechanism that it is surprising to find controversy on the general principle still occurring.

Adaptation is characteristic of the higher taxonomic categories, but is not always so easily demonstrated in species and subspecies, and certainly all specific characters do not seem to be functional. The explanation may be that the more adapted species survive and may then speciate further with the concomitant extinction in time of the less adapted species which have speciated through neutral variation and isolation. Also, as more adapted groups arise, they form the focal points for adaptive radiation into niches occupied by less efficient types.

[25] S. Wright. In J. Huxley, "The New Systematics." Oxford. pp. 161–183, 1940.

Noble,[26] however, points out that the degree of adaptive modification to the habitat bears no relation to the degree of specialization attained in phylogeny and gives the case of the adjustments of a primitive frog (*Ascaphus truei*) to mountain streams as an illustration.

Although adaptation is usually the result of a great many factors operating over long periods of time on complex organic systems, the ecologist must needs demonstrate selection as a factor at the initial stages of evolutionary divergence if he is to understand the influence of selective factors relatively uncomplicated by other factors. Correlations between environment and functional characters beyond the probabilities due to chance are the usual data indicating the operation of selective pressures. Such statistical correlations show such characteristic patterns that they may be used in prediction. For example, it is possible on occasion to predict the discovery of a new species of a certain genus in a certain locality and possessing certain specific characteristics. Wallace, having found a number of mimicry pairs of species in two genera of butterflies (*Delias* and *Huphina*) on various islands in the East Indies, but having found only a single species of the mimicking genus (*Huphina*) on Timor, predicted that a new species of the model genus (*Delias*) would be found on that island having certain color characteristics. Thirty-four years later his prediction was realized.[27]

Survival statistics and toleration experiments may be resorted to for better correlations demonstrating the possible action of selective factors. Among some interesting examples of such an experimental approach are those of Carrick[28] on color adaptations of insects in Britain, Isely[29] on protective coloration of grasshoppers in Texas, Talbot[30] on humidity toleration of ants in the

26 G. K. Noble. ''The Biology of the Amphibia.'' New York: McGraw-Hill Book Co., xiii + 577 pp. p. 87. 1931.

27 F. A. Dixey, *Trans. Ent. Soc.* London, 1920: 208–211, 1920.

28 R. Carrick, *Trans. Roy. Ent. Soc.* London, 85: 131–140, 1936.

29 F. B. Isely, *Ecology*, 19: 370–389, 1938.

30 Mary Talbot, *Ecology*, 15: 416–439, 1934.

Chicago region, and Hiesey, Clausen, and Keck[31] on climatic survival of different ecotypes among plants.

Occasionally experiments with excellent control over many factors have been performed in nature or unwittingly through human agencies. Such a case is to be found in the distribution and survival of the house rats. The Black Rat originated in the tropical orient and spread into northern Europe in medieval times. In the eighteenth century it was largely displaced by the Norway Rat which originated on the temperate steppes of Asia. Distributed by commerce, these two allied species have been and are being introduced to all parts of the world, but the Black Rat has established itself in the tropics while the Norway Rat has survived in temperate regions such as northern United States and Argentina. In competition, these species demonstrate their adaptation to the climatic conditions of their original areas. Without competition, the tropical Black Rat could and did overrun the human habitations as far north as Upsala, Sweden, where it was described by Linnaeus.

The ecologist studying adaptive similarities becomes aware very soon of functional similarities showing correlations with the environment but not with the taxonomic and phylogenetic patterns. Convergent evolution quite clearly shows how environmental selective pressures may move different organismic systems toward striking analogous functional resemblances. Cases may be cited all the way from class characters such as the wings of insects and birds or the eyes of vertebrates and squids to the species and race characters of black rodents of different genera on the black lava flows of New Mexico[32] and the remarkable locomotion of the Sidewinder (*Crotalus cerastes*)[33] and the African viper (*Cerastes vipera*) on the loose sands of the California and Sahara deserts.[34]

The positive action of selection of genetic variations

[31] W. M. Hiesey, J. Clausen, and D. D. Keck, AM. NAT., 76: 5–22, 1942.

[32] S. B. Benson, *Univ. Calif. Publ. Zool.*, 40: 1–70, 1933.

[33] W. Mosauer, *Ecology*, 16: 13–27, 1935.

[34] W. Mosauer, AM. NAT., 64: 179–188, 1930.

seems to be by far the best explanation of the origin of both endo- and exo-adaptations, but certain cases involving degenerative evolution have been more difficult to explain. Such degenerative organs as vestigial eyes are characteristic of cave species showing the same type of habitat correlation as adaptive characters, and yet such vestiges in themselves are hardly adaptive. However, modern genetics is able to supply a satisfactory explanation of such evolutionary trends. Several experimentally demonstrated principles may be assumed. (1) Each gene or genetic factor effects many characters. (2) Each character is effected by many genes. (3) Mutation of any single gene may occur at a statistically predictable rate (mutation pressure). (4) The effect of a mutation on a functional character is almost always deleterious or degenerative. (5) Selection acts upon the whole organismic unit and not alone upon the parts or definitive characters. (6) Elimination or weakening of a selective pressure will in time result in the degeneration of the functional character through the action of mutation pressure. It thus follows that if selection favors an increased development of one character and another character has a diminished survival value in a given ecological situation, there will be a shift in the alleles in many series with a consequent degeneration of the character losing importance.[35] Through the action of these tendencies, we have a reasonable explanation of non-functional vestigial structures and recapitulative phenomena.[36] We may thus understand why the eyes of many unrelated species confined to the cave environment undergo convergent degeneration in correlation with the functional enhancement of tactile organs or other useful characters, and at the same time understand why vestigial eyes are quite commonly present in these blind species. The complex genetic system correlated with an ancient and important adaptive character which has lost

[35] S. Wright, in A. E. Emerson, *Ecol. Monogr.*, 8: 247-284, 1938.
[36] H. V. Wilson, Am. Nat., 75: 20-30, 1941.

survival value in its present environment could not be eliminated suddenly without effecting many other vital characters. The universality of evolutionary degeneration of some ancient exo- and endo-adaptations in every organism may then be explained without recourse to Lamarckian mechanisms unsubstantiated by experimental data.

Intraspecific adjustments which adapt individuals of different generations to different environments (cyclomorphic populations) or adapt the individual to other individuals within the population are results of complex evolutionary processes. Aggregated populations of organisms have recently been experimentally investigated by leading ecologists, particularly by W. C. Allee, T. Park, and their associates. As a student of social insects, I am particularly interested in this phase of ecology and find it of considerable importance in the clarification of certain evolutionary principles. Not only is an analysis of integrative factors of populations from simple aggregations to complex societies of importance to the ecologist who is constantly confronted with these phenomena in the field, but the evolution of cooperative systems leads us to a better understanding of evolutionary mechanisms, ecological communities and human societies.

That various levels of aggregated populations show adaptation has been demonstrated by Allee[37, 38] through differential survival values of different numbers in toleration experiments on simply aggregated groups, and my own studies of the adaptive characteristics of termite nests built by highly social populations.[39] Some of the most remarkable social adaptations are found among the slave-making species of ants. Convergence is also amply demonstrated in social groups. Even among the insects,

[37] W. C. Allee, ''Animal Aggregations.'' Univ. Chicago Press. 431 pp., 1931.

[38] W. C. Allee, ''The Social Life of Animals.'' W. W. Norton and Co., New York. 293 pp., 1938.

[39] A. E. Emerson, *Ecol. Monogr.*, 8: 247–284, 1938.

highly complex societies arose convergently several times, the most striking example being the similar organization of ants and termites, while the complex societies of insects and men are predominantly convergent and analogous. Some of the convergent analogues in the societies of insects and men are social integration through signals, chemical agents, and activity gradients; social symmetry and relatively stable patterns of organization; social dominance; replication of patterns (polyisomerism); functional specialization and division of labor of diverse societal units through classes or castes (anisomerism); ontogeny of social organization; phylogeny of the social system; social divergence and convergence; recapitulation during ontogeny; degenerative evolution and vestigial social characters; social variation and selection of social and familial units; and the dynamic maintenance of population equilibrium of integrated social units (logistic curve of population growth). These analogues not only show a remarkable similarity between insect and human social systems, but in many instances they are also attributes of sub-social populations and individual organisms. Particularly in the case of the insects, the convergence between the organismic coordination and the social coordination is so complete that one may speak of the society as a supraorganism guided in its evolution by many factors that have also guided the evolution of the individual organism.[40] Thus we may find social examples of homology, instinct, hormones, physiological gradients, various types of symmetry, mechanical reactivity, ecological orientation, sterile somatic differentiation, reproductive adaptations, periodicities, size control, defensive adaptations, physiological isolation, budding, and duplications, and all these analogues have highly similar correlated factors chronologically preceding the appearance of these characteristics. However, as can be well illustrated by the genetic determination of sex in the higher social animals, homolo-

[40] A. E. Emerson, *Amer. Midland Nat.*, 21: 182–209, 1939.

gies are between the solitary organism and the individual within the society showing that the social supraorganism is an aggregation of individual organisms and not an extension of the organismic homologies of the individual.[40]

In spite of many common analogous attributes, human society shows fundamental differences from insect social systems and these must also be analyzed. Social insects are not separated from solitary insects by a striking quantitative differential in learning capacity. Also leadership as a result of conditioning is not as apparent as among men and the higher vertebrates. Correlated with these generalizations, human social evolution has taken place with great rapidity compared to the slow evolution of insect societies. We also find such human social systems as political government, law, police, educational institutions and religion lacking among the insects.

The basic reason for such differences may be explained by the addition of a new type of hereditary mechanism. Besides the usual biological heredity through genic patterns in the chromosomes which determine relatively stereotyped development by means of enzyme chains, the human species has developed a new mechanism which we call social heredity or culture.[41] This outstanding human attribute forms the valid division line between social and biological sciences. Man has symbolized his experiences and values by means of money, tools, pictures, and language, and these are transmitted through exchange, gestures, talk, writing, drawing, photography, printing, telegraph, telephone and radio, not only to his immediate associates, but to all parts of the world and to all succeeding generations. Thus our social patterns are repeated without involving the germ plasm and, in order to evolve socially, we do not need to wait for the genes to undergo favorable mutation and for the slow process of natural selection to sort out beneficial gene effects. Correlated with this additional mechanism of evolution,

41 A. B. Hollingshead, *Ecol. Monogr.*, 10: 354–366, 1940.

however, we are dependent upon a long individual life cycle and a long individual process of learning. Each human must spend a third of his life at least acquiring certain effective social traits and we must also train a large proportion of our population to function mainly for social coordination. What is most obvious at the present time, although the larger interdependent, international, world society is already highly integrated through commercial, transportative and communication systems, we have not yet been able to adequately master destructive national and class competition. It is our hope that the discovery by the social scientists of the mechanisms and details of cooperative social organization will ultimately enable mankind to evolve beyond this present phase with its inefficiency and misery. Within insect societies social intraspecific class or colony wars are practically nonexistent and this difference from men is correlated with the slow evolution of germinal heredity of social traits among insects and the rapid evolution of social heredity through symbols and learning among men. It is to be expected that our aggressive tendencies will be directed into constructive channels leading to greater social and biological efficiency in time.

The basic forces which have led to convergence in social patterns of insects and men and the parallelisms between individual organisms and societies remain to be explained. We have seen that the ecological principle of natural selection has guided evolution into adaptive channels. We have also noted that environmental fluctuations are limiting factors in the distribution of species. Not only do organisms acquire energy, reproduce their kind and defend themselves against predators and parasites, but they maintain themselves in the environment to which they are adjusted. This necessity for maintaining ecological position is seen particularly in the myriad adaptations for attachment and locomotion. Ecological position not only involves a relatively uniform physical environment, but also a relatively stable source of energy,

a relative security from enemies and a relative accessibility of cooperative individuals. Competition within favorable habitats has produced a selective orientation toward more efficient adaptations to the physico-chemical and biotic factors in the environment. In addition, adaptive radiation from more favorable primitive environments has occurred in all the main plant and animal stocks. Because of competition for limited necessities within favorable environments, there is an added selective force favoring mutations which would lessen this competition.[42] Most often adaptive radiation occurred by means of an internal control over an external fluctuation. Living protoplasm maintained a relatively constant composition of water, ions and metabolic materials in the face of environmental fluctuations of these materials. The naked cell, however, lived in less optimal conditions than did the cell in a group. Through division of labor and coordination between cells, the external environment of the single-celled organism became the internal environment of the multicellular organism. The evolution of the controlled cellular environment has reached a truly astonishing development of stable equilibrium and controlled periodicity in the physiological constants of higher plants and animals.

In spite of such successful evolution, however, there were still fluctuations in the external environment of the multicellular organism that seriously limited its existence. Aggregating forms could bring the external environment under partial control. Two trends of population evolution emerged in the development of greater living efficiency by means of natural selection. Ecological communities developed with interspecific integration which produced a partial environmental control and a relative environmental stability for the individual organisms. In addition to this type of organization, a second evolutionary trend directed the development of intraspecific popu-

[42] G. K. Noble, ''The Biology of the Amphibia.'' xiii + 577 pp. p. 83. New York: McGraw-Hill Book Company, 1931.

lation groups and progressed toward greater and greater
control of an otherwise fluctuating external environment.
Sexual adjustments brought a balance between hereditary
conservativism and variation. Reproductive adapta-
tions tended to control the environment of the young
during early developmental stages (*i.e.* the egg and the
uterus). Familial integration controlled the environ-
ment during the later stages of ontogeny (*i.e.* nests,
parental feeding, parental defense) and the family as a
biological unit of great importance became established.
Selection in these cases was not working alone upon the
individual organism. The population was the unit of
selection, much as the population of cells composing the
multicellular individual was selected as a coordinated
unit. A logical explanation of · the evolution of such
familial adaptations as mammary glands is impossible by
means of natural selection of individually fit multicellular
organisms. The family unit thus has attributes of the
organism for the simple· reason that the basic forces
guiding the familial and organismic integration are
similar.

But the end was not reached in the family organization.
More complex units could more thoroughly control the
external environmental fluctuations. Nests and shelters
could be made more adequate through the cooperation of
many specialists, enemies could be more successfully
repulsed, food could be brought under cultivation (*i.e.*,
fungus-growing social insects and human agriculture),
reproduction could be more adequately adjusted to the
population needs, hostile environments could be invaded.
Men and insects have accomplished these feats through
societal systems and the fundamental forces which have
guided social evolution have some identity even though
the germinal stocks are widely divergent.

Competition plays a tremendously important rôle in
evolution, but the survival of the fittest does not always
mean the survival of the strong, the predators, the para-
sites, or even the adequately defended organisms. Fit-

ness may mean cooperation for mutual benefit both
between species and within integrated intraspecific popu-
lations as well as between parts of the individual
organism. Cooperation between the parts of all living
organismic units is a fundamental biological principle.
Cooperation is not an end in itself, but is the means to
the end. Cooperation is one of the important adaptive
mechanisms with survival value which results in the
attainment of an internal and external equilibrium.
Natural forces guide the biotic unit toward the control
of the protoplasmic environment which is brought closer
to the optimal point for the organism concerned. The
optimum, with our present knowledge of physiology and
ecology, is often a rough approximation. Optimal con-
ditions at one stage of development are not optimal at
other stages. The optimum for one organismic function
is not necessarily the same for other functions. There
are regular and irregular cycles both in the organism,
supraorganism and environment which are reflected in
periodic phenomena, special adjustments and a com-
promise or balance between complex and sometimes
antagonistic forces. Balanced equilibrium is thus char-
acteristic of the units of each organismic level from
viruses and cells to the social groupings and ecological
communities and the division lines between these bio-
logical entities are not sharp.

The way in which the social supraorganism may inter-
digitate with the biotic community is illustrated by the
social parasites and commensals. Just as numerous
animal and plant species are incorporated into human
society, the social insect colony is an interspecific eco-
logical community consisting of numerous species of
plants and animals adjusted both parasitically and sym-
biotically to the internal environment of the supraor-
ganism. Cleveland[48] has shown that the wood-eating
roach and termite communities were fundamentally

48 L. R. Cleveland, Mem. Amer. Acad. Arts and Sci., 17 (2): x+1–342,
1934.

functional adjustments promoting an efficient cooperation between the wood-eating insects and their symbiotic cellulose-digesting intestinal protozoa. In order that the molted individual could become reinfected with protozoa, it was necessary for such an individual to live in a family or social community. Thus evolution has resulted in an integrated, balanced, biological system incorporating organisms of various species and various organismic levels, in its entirety exhibiting dynamic equilibrium between its parts and with its external environment.

Of what value are such generalizations? It seems to me that significant relationships between diverse phenomena indicate more universal and thus more fundamental principles thereby enabling us to arrange biological attributes in a more logical and scientific order. Chronological order may help us to detect causative factors more easily and to predict events with greater accuracy. Factoral analysis and the observation of the activity of whole synthetic systems produces knowledge of the guiding forces in the world in which we live. As knowledge grows, however, we become more aware of the great gaps in our factual experience and classificatory systems. We thus become more sensitive to our ignorance and more energetic in the accumulation of pertinent data. Through the advancement of science we gain a greater control over our complex internal, external and social environment, thus finding ourselves in harmony with the forces of progressive evolution which have been directing life since its origin.

THE INHERITANCE OF EGG PRODUCTION IN THE DOMESTIC FOWL[1]

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THE problem of inheritance of egg production in the domestic fowl has attracted considerable attention, particularly because of the practical implications of the subject. As is the case with many other characters of economic import, the progress towards the solution of the problem has been rather slow. In fact, to date only two significant landmarks in the history of investigations in this field can be pointed out. The first of these is a series of papers by Pearl (1909 *et seq.*) which constituted the initial fruitful investigation of the question, while the second is the methodological innovation introduced by Goodale (1918). The voluminous literature on the subject built up since (*vide* Jull, 1940), deals with many important points, but practically in all cases they are elaborations and refinements of what may be called the Goodale principle.

MEASUREMENT OF THE ANNUAL RECORD

The first step accomplished by Pearl consisted of the recognition of egg production as a heritable character based on Mendelian segregating units. Because of the comparatively low standard of productivity of most flocks at the time of Pearl's work, he found the egg production during the winter months to be a reliable criterion of the inherited ability to produce eggs. At the present time, however, winter production can no longer be used as the sole differential between superior and inferior layers. The distribution of egg production during approximately the first 18 months of life is considered a more adequate

criterion of measurement of laying ability. In breeding practice, the production during the 365 days immediately following the first egg laid by a bird is often held to be synonymous with the annual record. This period based on a temporal scale is, of course, entirely arbitrary from the biological point of view. The biological laying year used to designate the period of time from the first egg to the first complete molt has probably a better foundation, but it presents some difficulties in practical application. The difficulty lies in the fact that annual replacement of yearling hens in the laying houses by pullets coming into production has to be made, for the sake of efficient management, not individually but *en masse*. Consequently, the birds with extremely long biological laying years may be removed before molting, so that their biological laying-year records remain incomplete. As a compromise measurement the production from the date of first egg to a fixed date in the fall of the second year of life is often used. In the material presented in this paper, for instance, production to October 1 of the second year of life is referred to as the annual production.

The Components of the Annual Record

The contribution made by Goodale (1918) consisted of breaking down the annual record into its component parts. He considered annual production as an integration of a number of characters. Similarly, Hurst (1921) a few years later dissected the annual record into fall, winter, spring and summer production and attempted to assign special Mendelian factors for each. Such procedure not only involves arbitrariness of selection of seasonal limits but can readily resolve itself into absurdity should the process be extended by further subdivision into still shorter periods until oviposition on each day of the year is considered to be determined by the presence of a specific gene.

Goodale's original premise, on the other hand, is very sound. Though not stated in precisely this manner, the

concept expounded by him visualized annual egg production as a function of the time during which the bird is laying and of the rate at which the bird lays during that period. The time factor itself can be resolved into the time of commencement of egg production (age or date at sexual maturity), the time of onset of the molt which normally is coincident with cessation of production (age or date at last egg or persistency) and any periods of interruption of lay between the two above points on the time scale (winter pause or pullet fall molt, broodiness). By using this principle, selection for the total number of eggs produced in a year could be supplemented by selection against deficiencies with respect to these component characters so that the lack of desirable characteristics in one mate could be compensated by a specific superior trait in the other.

The formulation of Goodale's hypothesis was shortly followed by attempts to place the inheritance of these characters on a simple Mendelian basis. Hays, in a lengthy series of publications (1924 *et seq.*) suggested, on empirical grounds, that one or two genes are involved in the genetics of each of the following traits: (1) age at sexual maturity, (2) winter pause, (3) broodiness, (4) persistency, and (5) rate. Most of the other investigators have not entirely adopted Hays' interpretation, since it was obvious that the genetic picture had been greatly oversimplified. For instance, Hays himself was not able to eliminate all the undesirable recessive or even dominant characters from his flock after 16 years of selection (Hays and Sanborn, 1934), which should have been a comparatively simple procedure were only one or two genes concerned in their expression. However, the best that has been offered in lieu of Hays' theory was to invoke a larger number of genes responsible for the expression of each of the component characters. Munro, Bird and Hopkins (1937), at least by implication, rejected Goodale's principle altogether and postulated instead the existence of several hundred "protective" genes enabling birds pos-

sessing them to withstand adverse environment to produce the given number of eggs.

The fundamental misconception in dealing with Goodale's principle has been the assumption that the component characters are genetic entities, with a smaller or larger number of specific genes exercising an effect on their expression. An alternative point of view is that the characters contributing to the egg record are not necessarily independent phenotypic expressions of particular genes. Thus it may be suggested that such characters are purely arbitrarily defined observables which in combination account for the variation between birds in their annual production.

This point of view appears to be biologically sounder than that of Hays and is just as fruitful from the standpoint of practical breeding accomplishments. Any number of characters may be selected, so long as they answer three criteria:

(1) The characters chosen must contribute to the variance of the annual egg production.
(2) They must preferably be independent of each other.
(3) They must show genetic variability.

The definitions and methods of measurement of the characters chosen may be modified so as to maximize their contribution to the annual record and their genetic variability and to minimize interaction between them. It has been the effort of several investigators, whether or not they held to the point of view expressed here, to determine what particular characters answer best the criteria propounded. Gradually characters suggested by Goodale have been defined and re-defined until the first two criteria have been satisfied. The purpose of the present contribution is to examine these characters with respect to the third criterion, that of genetic variability and thus to complete steps necessary for the formulation of a theory of inheritance of egg production built' upon Goodale's original premise.

STUDIES OF THE COMPONENT FACTORS

Numerous reports are available on the correlation between annual egg production and each one of the component factors. For instance, with respect to maturity, Knox (1930) review 18 separate papers demonstrating the influence of sexual maturity on the variation in the egg record. The correlations between the other components and annual production have been similarly investigated, though not on as extensive a scale (*e.g.*, Hays and Sanborn, 1927; Knox, Jull and Quinn, 1935; Lerner and Taylor, 1937a). The magnitude of the contributions made by each of the factors varies, but there is little doubt that maturity, persistency, pause, rate and broodiness all contribute to the annual record. The way in which these factors are measured, of course, determines to a considerable extent the size of the correlation coefficients reported. The total amount of variability in the annual record accounted for by the variation of the components has been reported by Hays and Sanborn (1927) as about 75 per cent. by Lerner and Taylor (1937a) as 55 to 90 per cent. (only maturity, persistency and rate being considered), and by Knox, Jull and Quinn as 70–80 per cent. (including date of hatch as an additional independent variable).

The second criterion cited, that of independence between the measurements of the component factors, has proved a stumbling block for a considerable time. The first elaborate attempt at re-definition of Goodale's original variables was made by Knox, Jull and Quinn (1935) who, by using what in effect amounted to coefficients of alienation, determined which combination of individual measurements gave the highest multiple correlation with egg production. Their definitions, however, were not entirely adequate. For instance, rate was expressed by them as the percentage of production to March 1, a measurement which in reality combines two independent characters: net rate and winter pause (Lerner and Taylor, 1936). However, as a result of these and other studies it is possible to define at least tentatively the com-

ponents involved (the years refer to the publications by the present writers):

(1) Maturity—age at first egg (1937b);

(2) Persistency—date of last egg (1937b and c);

(3) Rate—per cent. production, calculated by excluding pausing and broody days from consideration (1936);

(4) Winter pause—presence before March 1 of non-productive periods of seven or more days' duration (1939);

(5) Broodiness—manifestation of broody behavior.

The third criterion noted refers to the genetic variability in these characters. That some degree of hereditary control is involved in all of these has been shown on many occasions. A single reference may be mentioned for each factor: maturity—Warren (1934); persistency—Lerner and Taylor (1937a); rate—Hays (1932); winter pause—Lerner and Taylor (1939); and broodiness—Hays (1940). However, no estimates of the actual degree of heritability have been made heretofore. The present paper makes an attempt to fill this gap in our knowledge of the subject.

MATERIAL AND METHODS

The material used in this study was obtained from the March- and April-hatched Single Comb White Leghorn flock of the University of California, including birds selected for and against desirable production characters as well as those selected for susceptibility to disease, size, differential shell thickness and other factors not immediately connected with annual records. Four series of pullets hatched in successive years were used, and in each all full-sister families of four or more birds were selected. A further prerequisite for inclusion in the analysis was that the dam and the sire were to have been hatched in the same year and to have a minimum of four full sisters also hatched in that year. This method of selection provided 117 families of daughters, including 905 pullets, which originated from 36 different sires and 109 different dams. A total of 1,480 females, including daughters, dams and the sisters of parents, were used in all. This figure does not include the cases of duplica-

tions where the daughters of one series were dams or aunts in another. There were five series of birds all told, since the earliest series contained only the sire's and the dam's families and the latest series only the daughters.

A number of statistics for each of the families were calculated. At the same time the constants for the same variables for all females hatched in each year were computed (Table 1). The raw data for each family were

TABLE 1

PRODUCTION CHARACTERISTICS OF THE BIRDS USED

Series	Total number of birds	Median age at first egg, days	Per cent. non-pause	Per cent. high rate	Per cent. persistent	Per cent. viability	Average maximum egg size, grams	Average production of survivors, eggs	Production index, eggs
K	230	187.2	43.2	47.1	61.8	48.7	58.5	238.5	154.7
L	305	180.3	33.0	57.0	63.8	59.7	60.7	226.5	166.4
M	433	176.3	35.7	56.7	40.0	49.9	60.0	223.9	154.3
P	299	176.8	29.6	55.8	44.1	36.8	58.4	217.6	129.6
R	213	172.9	20.1	74.4	55.8	58.2	60.6	235.8	176.3
Total	1480	177.5	32.7	58.0	51.9	50.3	59.7	227.8	155.0

then converted into deviations from these constants for the respective year of hatch. In this manner, environmental inter-year variation to which all birds hatched in the same year were subjected was removed. Similarly, the effect of any changes in the genetic make-up of the population between years was excluded. Since the members of the different families were not segregated at any time since their emergence from the incubator, it follows that the intra-year environmental effects are to be preponderantly found within rather than between families. The procedure used in the analysis deals exclusively with differences between families so that the intra-year environmental variation may be neglected.

The variables investigated, in addition to the component factors of egg production already enumerated, included such characteristics as egg size and viability, both important in commercial egg production. Broodiness was not included in this analysis, because its level has

been very low in the flock studied. Thus, there were eight factors not weighted for differences in the number of birds per family studied altogether:

(1) Sexual maturity, expressed as the median age at first egg for each family (Lerner and Taylor, 1940).

(2) Winter pause, expressed as the percentage of non-pausing birds (see definition above) in a family.

(3) Rate, measured by the percentage of birds in a family which showed a high rate of production, as judged by the records in the winter months (see Taylor and Lerner, 1938, for full description of this measurement).

(4) Persistency, expressed as the percentage of living birds in production on October 1 of the second year of life (Taylor and Lerner, 1938).

(5) Viability, as judged by the percentage of birds alive at approximately five months of age still living on September 25 of the second year of life.

(6) Egg size, expressed as the mean monthly maximum egg size for the family (Taylor and Lerner, 1938).

(7) Annual production of survivors to October 1 of the second year of life.

(8) Production index, representing the average number of eggs laid by each family on the basis of the number of birds housed at about five months of age.

DETERMINATION OF THE DEGREE OF HERITABILITY

The method best recommended for the determination of the degree of heritability is the intra-sire regression (Lush, 1940). However, it is not suitable for the material at hand, because in most cases here the characters dealt with have only a presence or absence value in the dam. Hence the following procedure was adopted. Simple correlation coefficients were computed between the values for the sire's sisters and sire's daughters, the dam's sisters (exclusive of the dam) and the dam's daughters, and between the values for the sire's sisters and the dam's sisters, again exclusive of the dam herself. In some cases certain of the families had to be omitted from consideration, where mortality did not permit some of the values to be calculated. For instance, if all the dam's sisters died before their persistency could be determined, the family had to be eliminated from the calculations involving persistency. In each case the daughters were included only where the values for sisters of both parents were available. In such manner the number of

families used for each factor varied from 100 to 117. The particular numbers, together with the correlation coefficients, are presented in Table 2.

TABLE 2
SIMPLE CORRELATION COEFFICIENTS

Character	Number of families	Correlation coefficients		
		Sire's sisters-daughters	Dam's sisters-daughters	Sire's sisters-dam's sisters
Maturity	117	0.295	0.168	0.087
Winter pause	116	0.184	0.172	0.260
Rate	113	0.253	0.290	0.372
Persistency	108	0.090	0.123	−0.011
Viability	117	0.257	0.012	0.158
Egg size	112	0.344	0.424	0.309
Survivors' production ..	100	0.293	0.231	0.247
Production index	117	0.294	0.233	0.384

The theoretical coefficient of correlation between individual aunts and nieces on the basis of random mating, without epistasis and $p = q$ with complete heritability, is 0.25 in the case of autosomal genes without dominance. With complete dominance the value of the coefficient under the same stipulations is 0.1667. In the case of sex-linkage, the sire's sisters-daughters correlation in either instance is 0.25, while that between dam's sisters and daughters is zero (Hogben, 1932 and 1933, making provision for the difference between mammals and birds with respect to the heterogametic sex).

On the basis of these figures it is easy to see that the determination of the daughters' values by those of the two types of aunts combined (the square of the multiple correlation coefficient) is 12.50 per cent. in the case of autosomal inheritance without dominance; 5.56 per cent. with dominance, and 6.25 per cent. in the case of sex-linkage with or without dominance.

Under conditions of random mating the comparison of the observed figures with these theoretical figures should indicate the degree of heritability[2] of the characters in

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[2] The degree of heritability as used here refers to that portion of the variance between families which is attributable to genetic effects. It should be clear that the degree of heritability between individuals in Lush's (1940) sense is higher than our figures, because it includes the intra-family genetic variation as well as the inter-family genetic variation considered here.

question.   However, the data used here do not consist of
individual values but of averages.   Dr. J. L. Lush has
pointed out to us that the effect of averaging is to in-
crease the theoretical correlations.   Because of the effect
of the correlation between full sisters (both in the aunt
family and in the niece family), the aunt-niece average
correlation in the extreme case, that of autosomal inheri-
tance without dominance, approaches 0.50 as a limit as
the size of the families increases.   Under these condi-
tions the theoretical multiple determination rises to 50
per cent.   As indicated in Table 2, most of the matings
in the material at hand were assortative to some extent.
There is, however, considerable variation in this respect
in the different characters.   The maximum degree of
consanguinity represented is in the case of two full
brother × sister matings out of a total of 117 matings.
In order to calculate the increase in genetic variability
due to the non-randomness of matings, Wright's method
of path coefficients as outlined by Tolley (Elliott, 1927)
was used.

The Degree of Heritability of the Component Factors

Table 3 presents the results of this analysis, showing,
in successive columns, the total determination of the
daughters' values by both types of their aunts, by their

TABLE 3
Coefficients of Determination*

1	2	3	4	5
Character	Total determination (R^2)	Direct determination by sire's sisters	Direct determination by dam's sisters	Joint determination
Maturity	0.1075	0.0798	0.0207	0.0070
Winter pause	0.0503	0.0223	0.0176	0.0104
Rate	0.1089	0.0284	0.0519	0.0286
Persistency	0.0236	0.0084	0.0154	− 0.0002
Viability	0.0669	0.0684	0.0009	− 0.0024
Egg size	0.2293	0.0554	0.1225	0.0514
Survivors' production	0.1126	0.0631	0.0285	0.0210
Production index	0.1033	0.0576	0.0199	0.0258

* R^2 at $P = 0.05$ varies from 0.0515 to 0.0625, depending on the number of families.

paternal aunts directly, by the maternal aunts directly, and the joint determination. It may be seen that with the clear-cut exception of persistency and the border-line case of winter pause all of the total figures are significant. In comparing the relative contributions of the sires and the dams to the variability of the daughters, it is possible that previous selection has had a differential effect on the variability of the sisters of each of the parents. There is no reason to suspect, however, that in this population such effect would be of any great significance.

In the case of maturity, the operation of sex-linked genes is suggested by the considerable excess of the figure in column 3 over the one in column 4. It is highly likely that similar excesses for the survivors' production and the production index are due to the sex-linked genes for maturity. These have been noted earlier by Warren (1934) and by Hays (1936) within the Rhode Island Red breed.

The contributions made by the paternal and maternal aunts to the winter pause of the daughters are about equal. In the case of rate, the determination on the dam's side is somewhat greater than on the sire's side, but not necessarily significantly so. The measurement used for persistency does not appear to represent adequately any of the possible genetic variability in this material. The reason for this is obscure, since date of last egg has been shown previously (Lerner and Taylor, 1937b) to exhibit significant variability between sires. Neither is it clear why viability of the dam's sisters is not correlated with that of the dam's daughters. The two measurements of production (survivors' production and production index) are the result of integration of the first four and five characters respectively. Since they do not operate in a direct additive fashion and, in fact, may in some instances run counter to each other in their effect on egg production, the total determination in the case of the latter is not necessarily equal to the sum of the determinations of the component characters. The

value reported here for survivors' production approximates rather closely the estimate of the proportion of genetic variability in records of Canadian egg-laying contests made by Munro (1936).

The considerable excess in column 4 over column 3 in the case of egg size might indicate either a maternal effect or an effect due to factors carried on the heterochromosome. The two possibilities can not be distinguished from the material at hand. It is curious that indications of a similar effect have been noted with respect to some other egg characteristics such as shell thickness (Taylor and Lerner, 1939) and percentage of firm white (Lorenz and Taylor, 1940).

For the purpose of estimation of the degree of heritability the figures in column 2 of Table 3 may be divided by one of the theoretical figures cited above. To be on the conservative side the minimum degree of heritability may be determined by using the highest of these as the denominator. This is the aunt-niece correlation on basis of autosomal inheritance without dominance, assuming the number in each family to be indefinitely large (0.50). This would obviously lead to an underestimate of the degree of heritability, since (1) the number per family averages less than eight, (2) there are sex-linked factors involved in some cases, and (3) some of the genes may be dominant. The quotients thus obtained appear in Table 4. They represent then the minimum heritability in this

TABLE 4

Minimum Estimates of the Degree of Heritability between Families

Character	In actual population, per cent.	Under random mating, per cent.
Maturity	21.5	20.1
Winter pause	10.1	8.0
Rate	21.8	16.1
Persistency (not significant)	4.7	4.8
Viability	13.4	13.9
Egg size	45.9	35.6
Survivors' production	22.5	18.3
Production index	20.7	15.5

population. It is also possible to estimate the degree of heritability were random mating practiced. The assorta-

tive mating in this population is largely due to the formation of non-interbreeding families. This, of course, increases the proportion of genetic variability between families. In random matings the joint contributions would not appear and division of the sums of the direct contribution by the theoretical multiple correlation would give the desired data, also shown in Table 4.

CONCLUSIONS

It is clear from these data that in the flock studied there still is a reserve of genetic variability in all of the characters studied, with the exception of persistency as here measured. This reserve is of sufficient magnitude to make selection on the basis of progeny testing an effective tool in improving production characteristics (see Wright, 1939). In the case of persistency, it is possible that a different measurement than the one used here need be selected.

It may be then considered that the present paper demonstrates the application of the third criterion to the Goodale principle. On the basis of the data presented here, and those referred to in the course of the discussion, it is possible to state that egg production records are resolvable into independent observables with high genetic variability significantly contributing to the variation in egg production. It is obvious that observables such as these may be selected arbitrarily for any other production complex as well. For successful and efficient application in breeding practice they have to answer the criteria listed, and so long as they do, can be used advantageously in improvement programs, irrespective of their biological basis.

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SPECIALIZATION AND EVOLUTION

DEAN AMADON
AMERICAN MUSEUM OF NATURAL HISTORY

HIGHLY specialized animals have always aroused man's natural interest in the bizarre and unusual in nature. Biologists when writing text-books and popularizations have utilized this interest, witness the frequency with which dinosaurs, birds of paradise, giraffes, whales and other remarkable animals are figured and mentioned in such works. The role of such specialized forms in evolution has also received much attention. Most zoologists seem to be in agreement concerning this question. Their conclusions may be summarized briefly as follows: although specialized animals are interesting and, in the case of such forms as the neutral castes of social insects, almost baffling examples of the potency of natural selection and other evolutionary factors, they represent extreme end-products of evolution which have or will become extinct more quickly than their less specialized relatives whenever conditions change, however slightly. For this reason it is generally believed that specialization has been of little importance in evolution, since during periods of geologic upheaval and great environmental change only generalized forms have survived. Furthermore, the belief is widely held that specialization runs its course, so to speak, and that specialized forms become, as it is variously expressed, "senescent," "implastic," "static," "over-specialized" or "genetically fixed" and hence incapable of further change or adaptation or of playing a further part in evolution. This line of thought is apparent in the frequently encountered assertions that man as a species will have a short future history because he is too specialized for further extensive genetical modification or evolution to be possible. The present note is written to present the opposite point of view, namely, that many of the most important advances in evolution have been the

result of specialization and that modern knowledge of genetics suggests that specialized animals are in general even more likely to undergo further genetical modification than are unspecialized ones.

Specialization and Survival

We may define a (morphologically) specialized animal as one having one or more organs or structures greatly modified or developed as compared with other related species. Sometimes an entire group is specialized and comparison must be made between groups. As regards probability of survival under changed conditions, it is possible to distinguish two types of specialization. The first is that found in a species which is specialized in such a manner as to make its existence dependent upon the continuance of a peculiar environmental niche or sequence of events. The inter-dependence of the yucca moth (*Pronuba*[1]) and fig wasp (*Blastophaga*) with the plants which they pollinate are classic examples. If either plant or insect were to become extinct, the other member of the partnership would become a victim of its own specialization. Host specific parasites, especially those which pass successive stages in different hosts, would also seem to be specializing in a way which imperils their survival.

Consideration of such examples as the preceding is responsible for the belief that specialization *per se* leads to an evolutionary *cul de sac* and eventually to extinction. Such a conclusion, however, ignores the numerous forms which are equally specialized morphologically and yet are as well or better equipped for survival than more conservative species. The extreme specialization of whales has permitted them to utilize an environment which is more widespread and less subject to change than the habitats of terrestrial mammals. Birds, as Huxley remarked, are only "glorified reptiles," but they seem better able to survive than their less "glorified" reptilian relatives. Relic survivors of nearly extinct groups are often highly

[1] Now *Tegeticula.*

specialized.. In some cases at least, it seems probable that . such species have survived because they were specialized. This would seem to be true of the mono-tremes and many primitive insectivores which are highly specialized for unusual aquatic or subterranean niches. Simpson (1941, p. 17) has written of the king-crab, a member of a group which has flourished almost unchanged since the Triassic, "Far from dooming it to extinction, its specializations seem almost to be a recipe for group immortality." Nor can it be assumed that major changes of environment will necessarily place specialized forms as a group at a disadvantage. Bats and birds, for exam-ple, would probably find the mobility which specialization has conferred upon them advantageous under such condi-tions. Even of species which seem to be jeopardizing their survival by specialization for restricted or marginal habitats, a certain number would probably be benefited by changes of environment. A catastrophic rise in tem-perature, for example, might be fatal to all life except those algae which are specialized for life in hot springs.

SPECIALIZATION AND EVOLUTIONARY ADVANCE

One of the puzzling features of historical evolution has been the apparent suddenness with which many major groups seem to have appeared and the correlated rarity of "missing links." So formidable has this difficulty appeared to some students that they have concluded that new species and even major groups appear instantane-ously as the result of radical "systemic mutations." Such a point of view is at variance with the known facts of geographical variation and genetics and introduces a pre-Darwinian mystical or miraculous aspect into evolu-tion. Is it not possible that the difficulty arises from the fact that new major groups have often evolved from highly specialized (and hence often rare) forms? The rarity of missing links and the apparently great gaps which separate many major groups are both understand-able if it is assumed that the transition from one major

group to a derived one is often effected by one or a few very aberrant and specialized forms.

The discoveries of paleontologists have supplied much evidence which seems to support this suggestion. It was, for example, a very specialized group of fishes which acquired the ability to breathe air and walk on their fins and thus gave rise to the amphibia and eventually to all the higher vertebrates. By way of contrast, sharks, a generalized and conservative group, have not changed greatly since the Devonian except to degenerate somewhat. Again, one specialized branch of the Reptilia gave rise to the birds and another to the mammals. Meanwhile reptilian groups with less of a tendency towards specialization, such as *Sphenodon* or even the more primitive families of lizards, have remained essentially unmodified. Two of the most specialized orders of mammals, bats and whales, must have evolved as a result of specialization which continued until the attainment of flight in the bats and independence of land in the whales suddenly provided "new worlds to conquer." Although at that time the ancestors of both groups must already have been highly specialized for a long period, there is no evidence that this had reduced their potentialities for further modification and evolution. Instead both underwent a rapid adaptive radiation until the opportunities of the new medium had been exploited.

An illustration of how specialization might again lead to an important evolutionary advance is supplied by Raven's recent studies (1939a,–b) of the anatomy of the peculiar ocean sunfishes of the family Molidae. In this family almost every structural feature of ordinary fishes has been transformed and specialized. These modifications have produced an amazing disc-shaped fish in which the *lateralis* musculature, which comprises 90 per cent. of the weight of a typical fish, has been completely lost. The locomotor function usually performed by these muscles has been taken over by the erector and depressor muscles of the dorsal and anal fins, which have become

greatly hypertrophied. Most interesting, then, was the discovery of a rare and most-modified member of the Molidae, *Ranzania truncata,* in which the body form has become secondarily elongated to again approach that of an ordinary fish. It is entirely conceivable that its new pattern of musculature might give *Ranzania* a competitive advantage over other fishes. If this should happen, many of the latter would be replaced by Molids, which would rapidly, phylogenetically speaking, differentiate into a variety of forms adapted to various ecological niches.

The foregoing considerations make it appear probable that it has frequently been specialized forms which in the past have and in the future may be expected to give rise to new groups of major rank. For it is only in highly specialized forms that the possibility exists of stumbling upon, so to speak, entirely new structures or processes, of which a few from time to time will prove to be of revolutionary importance.

SPECIALIZATION AND GENETICS

Evolution might almost be defined as a process by which originally simple organisms have become increasingly modified and complex in structure and function. Partial reversals of this trend may be observed in so-called degenerate forms, but nevertheless all the members of such advanced groups as insects or mammals are amazingly complex when compared with representatives of lower and broadly speaking ancestral Phyla. The implications of this trend, it would seem, are overlooked by those who believe that specialization leads to genetic fixation. Specialized forms are those in which the usual evolutionary trend towards increasing complexity has been somewhat accelerated. On *a priori* grounds it is more logical to suppose that they will continue to change faster than their more conservative relatives than to postulate the opposite.

Dobzhansky's recent summary (1941, especially Chaps.

5, 6, 10) of genetics as related to speciation and evolution gives no support to the belief that specialization decreases a species' potentialities for further change. He points out that each species (or more or less isolated population within a species), as a result of the selection exerted by the particular environment in which it lives, tends to reach an adaptive peak in which the effects of such dynamic factors as mutation pressure, population size and migration pressure are balanced. If any of these factors, including the environment, changes or becomes dominant over the others the rate of evolution will thereby become greatly accelerated or retarded. There is reason to believe that in highly specialized species such an unbalanced condition will exist more frequently than in others. Thus the total populations of specialized species will often be small, since they are frequently adapted to an unusual and restricted type of environment. In such populations, as Sewall Wright and others have proved theoretically, natural selection becomes largely non-effective. Favorable genes may be lost and neutral or deleterious ones fixed merely by chance genetical recombinations. The result is a non-adaptive population which is undergoing rapid genetic change. This will often lead to extinction, but sometimes one of the new genotypes, which has been produced by chance, will prove to be "pre-adapted" to a new, unoccupied environmental niche. When this occurs, an increase in numbers will follow and the modified species will reach a new adaptive peak adjusted to its changed habitat.

The acquisition of tusks in the Proboscidea may have resulted from the operation of such processes in a numerically small, specialized group. Apparently the tusks had their origin in a mutation which prevented occlusion of the incisor teeth. Although this mutation was probably at first unfavorable, at least one population in which it had become established through chance genetical fixation was able to survive. Eventually the tusks became useful in fighting and perhaps in feeding. They

then came under the influence of natural selection and finally only those species in which they were well developed survived.

It is possible that specialization tends to change the rate of evolution of a species in other ways also. Some have suggested that such groups as the birds of paradise, in which almost every species is characterized by peculiar specializations, may have acquired an unusually high rate of mutation by natural selection. The mating habits of this family confer an advantage upon specializations which permit females to immediately recognize males of their own species. Until more research has been accomplished in population genetics, it is not profitable to speculate further. It is to be hoped that students of this promising subject will give especial attention to the study of evolutionary patterns in highly specialized species.

SPECIALIZATION AND CLASSIFICATION

Some systematists have incorporated their beliefs on the role of specialization in evolution into the classifications which they have proposed for various groups. Wetmore, for example, in his revised classification of the Class Aves (1940) has placed the relatively generalized sparrow family at the apex preceded by more specialized groups because the latter are " . . . assumed now to be more or less static and fixed and therefore should stand at the side" (p. 2). However, if such a procedure were adopted for animal classification in general, it would be necessary to place the generalized protozoans, etc., at the apex, preceded by all the specialized, derived groups!

A classification can not express everything, and it is equally inadmissible to put a specialized group above others merely because it is specialized. The important thing is to put ancestral groups below derived groups which evolved from them. Some members of ancestral or lower groups may, of course, survive to exist side by side with the derived ones. The duck-billed platypus is a uniquely specialized mammal, but as regards those par-

ticular specializations of the pectoral girdle, reproductive system, etc., which led to all the modern mammals it is very generalized, almost reptilian. This shows it to be a surviving relic of a group ancestral to the marsupials and placentals and it is correctly placed below them in accepted classifications.

It is here assumed that a phylogenetic classification is the goal desired. Strenuous objections from time to time are brought against this assumption by those who believe that animals should be classified according to the totality of characters which they have in common, regardless of relationship. As Dobzhansky (*op. cit.*, pp. 362–365) has shown, this conflict is more apparent than real, first because phylogeneticists must perforce usually infer phylogenies from the possession of common characters plus (sometimes) sequence in time, and second, because the assumption that phylogenetically related animals will almost always have more in common than those not so related appears to be valid. It might be pointed out that this last assumption receives partial proof from breeding experiments which show that strains having close blood (phylogenetic) relationship are as a rule more alike than more distantly related strains.

Summary

Although specialization sometimes leads to extinction, it often confers an advantage upon a species in its struggle for existence. There is considerable paleontological evidence to indicate that new groups have often evolved from specialized members of a lower group. The suggestion that specialization has played an important role in evolution is not at variance with present knowledge of genetics, and receives some support from current theories of population genetics. The relation of specialization to classification is discussed.

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EDMUND B. WILSON—AN APPRECIATION

DR. H. J. MULLER

UNITING CYTOLOGY WITH MENDELISM

WILSON's remarkable appraisal of the problems confronting all research, which was reviewed in the preceding section, was published four years before the three almost simultaneous epochal announcements of the rediscovery of Mendelian heredity. The stage had indeed been set, and this discovery was no longer too far ahead of its time. Both Correns and de Vries very soon offered a chromosomal interpretation of their findings. This, however, was based on an assumed random assortment of conjugating chromomeres which—though it contained the germ of the crossing over theory—departed in some ways too far from the then ascertainable facts of chromosome behavior; consequently it did not serve as the main route by which the connection of cytology with Mendelism was made. It was reserved for Sutton, working as a student of synapsis under the guidance of Wilson, to confirm in his own preparations Montgomery's surmise of chromosome homologies, and by its means to show that the visible behavior of the chromosomes, so far as known, was such as to afford an explanation of both the first and second Mendelian laws—though the chromosomal application of the second law (that of random assortment) was as yet only a very probable inference, based on the visible separateness of the pairs. Wilson at once (1902) came to the support of Sutton's conclusions, while at the same time calling for more extended study of the cytological phenomena concerned.

In the same year McClung—who had started in cytology as a student of Wilson's only three years before—called attention to the fact that the unpaired chromosome (later called the "X" by Wilson) long known to exist in the males of some arthropods might provide an

explanation of sex determination. Confirmation of this penetrating inference was delayed by faulty observations of some other workers and also by apparent contradictions occasioned by McClung's having assumed that the X-containing sperm were male-producing. But in 1905 Stevens and Wilson both brought forward detailed cytological proof of the main principle—showing, however, that the X-containing sperm were female-producing, inasmuch as the female contained two X's. Thus the case for the chromosomes' constituting the basis of heredity no longer remained dependent upon the general parallelism between their mode of behavior and that of the Mendelian factors, as this line of evidence was now supplemented by the proof that given character-differences of prime importance were in fact bound up with the transmission of given chromosomes. In this way cytology became very definitely implanted into the science of heredity.

Stimulated by these findings, and being at the same time in both home and laboratory environments that were more than ever conducive to creative work, Wilson was now entering upon the years of his most productive researches—1905 to 1912. It was in this period that his series of eight classical "Studies on Chromosomes," based upon the intensive observation and analysis of a great array of exquisite preparations, mainly of Hemipteran spermatogensis, was published. In these "Studies" and in Wilson's accessory articles of the same period we find the working out of the whole general theory of sex determination, Mendelian in principle, that forms the basis of our modern conception of this subject. Wilson's comparative observations showed that, in different species, the heterogametic sex might have the X's and Y's equal or unequal in size, that one of the other members might be dual or even multiple, and that all gradations existed from Y's equal to the X through smaller and smaller ones down to the "XO" condition. On the basis of this evidence he postulated (1906 *et seq.*) that the Y had undergone a continued degeneration, and in 1909

(Study V) he adduced more definitive genetic evidence of
its being either inactive or representing "an excess of
chromatin that is duplicated elsewhere in the chromosome
group," in his finding of apparently normal females (as
well as males) in *Metapodius* having one or more super-
numerary Y's or Y-parts, and of males lacking a Y. In
the same paper (1909) he explained the origin of these
cases through failure of separation of the X and Y, an
aberration which he had observed to occur occasionally
in spermatogenesis, and he pointed out that this would
in fact be a method of origination of the XO type. When,
four years later, Bridges encountered the same phe-
nomenon in breeding experiments with *Drosophila*, he
and Wilson, in consultation together, agreed to term the
process "non-disjunction."

By the same evidence, Wilson showed that it is the X
which is the active chromosome in sex-determination, and
that it differs qualitatively from the Y—"perhaps as the
bearer of a specific substance (enzyme?) that calls forth
a definite reaction on the part of the developing indi-
vidual" (1909). Following up an earlier suggestion of
Stevens (1906), he further postulated (1912) that there
is still, in "X Y" forms, a part of the X more or less
homologous with Y, but that the sex-determining material
lies in the other part. This idea is surprisingly near the
truth as we now conceive it, except for the added sug-
gestion that the differentiating factors for sex-linked
characters lie in that part of the X homologous with
the Y.

Laying aside, after some consideration, the "unit-
character" idea of sex proposed by Castle, according to
which a given member of a chromosome pair carried
"maleness" or "femaleness" *per se* and these were
allelic characters for which both sexes were heterozygous
(a scheme that had the additional difficulty of requiring
selective fertilization), Wilson in 1909 developed further
his own view that the material of the X chromosomes did
not bear either maleness or femaleness in any absolute

sense but was of such a nature that, when acting in combination with the rest of the genotype, the amount present in one chromosome reacted to such a degree as to result in maleness, while that in two chromosomes, reacting more intensely, resulted in femaleness. This of course conformed just as well as Castle's scheme with the principles of Mendelian transmission—though that was realized by few Mendelians at first, and it provided just as critical an illustration of a segregating character whose distribution followed that of the chromosomes of a given pair.

The types in which the female is heterogametic in respect to sex determination were also brought into Wilson's scheme finally, but by a more roundabout route. It had been shown by Punnett and Bateson (1908) that the results of Doncaster and Raynor on what we now call sex-linkage in the moth *Abraxas,* as well as similar results in birds, were best interpreted on the basis of (1) the female being the sex-heterozygote in these cases (they did not regard it as surprising that in other large groups, on the contrary, as shown by Wilson and Stevens, the male was the sex-heterozygote), and (2) the existence of "repulsion" between the "factor for femaleness" and the dominant factor for the color character studied. They preferred not to interpret these phenomena in terms of chromosomes (partly because this would require the "dominance of an absence" in XO forms), but Spillman in the same year (1908) proposed this interpretation. Wilson, after having considered this and the somewhat similar suggestion of Castle's (1909) with reference to these types, accepted this converse sex-chromosomal scheme for these forms, on the basis of the evidence from sex-linkage. In the seventh Study (written in 1910, published in 1911) he gave diagrams of this and pointed out that the lack of XY figures in the males of all Lepidoptera and birds thus far examined was in good agreement with the conception.

A point of great theoretical interest in Spillman's de-

duction of 1908 (though one not emphasized by him) lay
in the implication that there are other Mendelian char-
acters, besides sex itself, dependent upon or linked with
the chromosomes of the sex-determining pair. Wilson in
his seventh Study stressed the importance of this and of
the essentially similar facts which had meanwhile been
discovered by Morgan in *Drosophila,* where—thanks to
Stevens—the cytological picture had already been worked
out. Owing to her having proved in 1908 that the *Dro-
sophila* male was heterogametic for the sex chromosomes
the mode of inheritance found by Morgan left no doubt
of the truth of the inference, proposed both by Morgan
and by Wilson in 1910 (see Wilson, 1914), that the domi-
nant sex-linked genes went with the X chromosome in its
segregation. Although for a time Wilson thought that
this linkage might be an expression of what is now called
"chromosome linkage," he soon came to see in it a cor-
roboration of the conception of the compoundness of the
chromosomes which he espoused (see below), as well as
of that of the multiple nature of the hereditary basis of
characters.

The investigation of sex-determination in itself was but
one aspect of Wilson's chromosome studies. He probably
regarded as of greater significance the evidence they gave
concerning even more basic problems, such as that of
chromosome individuality, or "genetic continuity," as
he preferred to call it. This was a principle still widely
contested, even by such leaders as O. Hertwig, and some
destructive critics, like Fick, were contending that the
chromosomes were nothing more than crystallization
products formed anew in each cell-generation from the
general nuclear material. In part, as Wilson and Stevens
both pointed out, striking evidence of chromosome indi-
viduality could be derived, secondarily,.from the facts of
sex-determination themselves. For, according to the only
scheme that worked, the egg with one X to begin with
that received another X from the sperm came to have
two X's and kept both of these throughout all its subse-

quent divisions up to the next reduction, while, *mutatis mutandis*, the egg that received a Y retained the XY combination throughout development. This would have been good enough in itself, but the facts found by Wilson went a good deal further. Thus, the considerable diversity of different individuals of the bug *Metapodius*, and of some other species, in regard to their number of chromosomes (referred to on p. 144), was combined with a constancy of the chromosome configuration throughout the unreduced cells of each individual. Hence, even in the case of unusual chromosome configurations, each of the chromosomes that had been received at fertilization (or a replica of it) reappeared after the successive resting periods during which it had seemed obliterated. Moreover, in these test cases where the configuration was aberrant, the chromosomes showed this persistency not only in their number and their individual morphology (size and form) but also in their behavior, for the extra Y's showed their peculiar reactions of remaining more condensed, undergoing late conjugation, etc. The most demonstrative case of this sort was supplied by an individual of *Metapodius* observed by Wilson in 1910, in which there chanced to be no Y, but three of the small "m-chromosomes," so that the total chromosome number was normal. Here it was evident that an *m* could not take the place of a Y, and all three *m*'s alike showed the peculiar meiotic behavior which Wilson had previously found to be characteristic of the two *m*'s of normally constituted individuals.

Going beyond the individuality of the chromosomes as wholes, Wilson adduced indirect evidence of their being compounded of parts each having their own individualities. This was largely derived from comparisons of related species, in which the X might appear as one entire chromosome or as more or less subdivided (though still of the same total mass, relative to the rest), or in which, in still other cases, a chromosome seemed to have had a portion detached that had become attached elsewhere.

Wilson concluded (seventh Study) that the exact conformation of the chromosomes as wholes was a relatively unimportant matter in the determination of characters, and that it could be changed by breakage, as well as by fusion of smaller parts in which the genetic continuity really resided. In some groups, he observed, the interspecific variability of the configurations was much greater than in other groups, and he was inclined to believe that in the latter cases wide-spread rearrangements might occur. By 1913 he was ready to assert that "microscopical investigation (independent of experiments in heredity) has made it almost certain that the chromosome is a compound body, which includes many smaller elements, . . . each of which may play a definite part in determination."

As pointing towards a possible basis for a far-reaching genetic continuity of the chromosomes, Wilson in 1909 and again in several later papers called special attention to the results obtained in 1908 *et seq.* by Bonnevie (who afterwards came to work with Wilson for a time), and later obtained by other workers. According to these, the prophase chromosomes, in rapidly dividing cells of widely different forms, could be seen each to consist of a coiled thread (later called the "chromonema"), that had been directly (or according to the earlier accounts "endogenously") derived from a similar coiled thread of a preceding telophase chromosome. In this connection, however, it should be noted that, after making careful observations of his own on the matter, Wilson continued for many years to regard this doctrine as far from established, and he still gave serious consideration to the earlier views of the proponents of chromosome individuality. According to these, the various differentiated portions of the chromosomes that are aligned in single file at mitosis become branched out (Boveri) or even to some extent diffused during the telophase and resting stages and later, during the early prophase of the next mitosis, become retracted again, if not somehow reformed, into their determinate mitotic positions.

A serious gap in the evidence for the genetic continuity of the chromosomes and their parts and for their constituting the basis of Mendelian transmission lay, in the extraordinary obscurity still shrouding the cytological phenomena in many objects during the stages of synapsis and reduction, where the events most crucial for genetics would have to occur. The very existence of conjugation of homologous chromosomes was still violently contested, and even where conceded was more often considered to be an endwise pairing (telosynapsis) than a longitudinal one (parasynapsis). The latter process, which had been described more especially by Winiwarter, Strasburger, Janssens and the Schreiners, seemed to be required on the chromosome theory of heredity if the then accumulating indications of the existence of more separable hereditary factors than chromosome pairs were trustworthy. For it would give opportunity for orderly interchange of factors, as proposed by de Vries, Correns and Janssens (see below). As yet, however, these proposals seemed on the plane of speculation. Taking the mass of destructive criticism seriously, Wilson reexamined the subject "in a distinctly sceptical spirit," and made a searching and objective investigation not only of preparations of his own but also of amphibian and annelid (*Tomopteris*) material borrowed from Janssens and the Schreiners, and some Orthopteran material from McClung. In his eighth Study (1912) he emerged convinced by this examination not merely that synapsis does occur, but that, in all the material observed by him which was suitable for throwing light on the question, it occurred by longitudinal pairing.

In the amphibian and *Tomopteris* material the Y figures formed by what we should now call the "zipper action" of the partners were plainly visible, and the consequent halving of the number of visible threads was made more apparent by the fact that at this time the stems of the Y's tended to lie parallel, resulting in a "bouquet" figure. There were indications of the same process in the Orthoptera (which McClung had thought

not to have parasynapsis), but the observations here were obscured by the lesser degree of parallel orientation. In Wilson's Hemipteran material the stage of synapsis unfortunately occurred just at the moment when nearly everything in the fixed specimens was rendered indistinguishable by a contraction ("synezesis") of the chromatin contents of the nucleus, but in the stages just before and after this the conformations were seen to be essentially like those in the material in which the process of conjugation could be directly observed. The sex chromosomes in the Hemiptera afforded evidence on the question from a different angle. Remaining in a condensed condition, they could not undergo a real parasynapsis, yet they retained their identifiability throughout and could be seen to show a distinct tendency to come together. The *m* chromosomes also were aberrant, in appearing as separate chromosomes during most of the synaptic period, yet they too later, when in a condensed condition, underwent a sort of touch-and-go conjugation that could be seen very clearly. Thus these chromosomes of special types, partly because of the very lack of intimacy of the process of synapsis in them, were unusually favorable objects for demonstrating both the persistence of chromosome individuality and the reality of the pairing process in general and of the following process of segregation of the partners.

As for the ordinary chromosomes, in the stage of "pachytene" (thick threads), immediately following their conjugation, visible evidence of a persisting duality of the threads could not be obtained and so the question of the degree to which the partners might have actually fused could not be solved by direct observation. Wilson noted, however, that the impress of their dual nature was evident in their "valence," as expressed by their later forming tetrads, in contrast to the behavior of chromosomes like the X and Y in much of the Hemipteran material, and the *m*'s. For the chromosomes of these special types, which emerged from the synapsis and pachytene

stages still recognizably separate, formed only diads, at least until the time of their delayed conjugation, in condensed condition. On the basis of this comparison, it was inferred that the ordinary chromosomes, which had appeared to unite completely in synapsis, nevertheless retained within them some features of their originally dual nature.

The above evidence did not mean for Wilson, however, that the chromosomes behaved as indivisible units in synapsis. For, following Boveri, de Vries, Strasburger and Janssens, he saw in the intimacy of the parasynaptic union of the more typical chromosomes an opportunity for some sort of exchange of material, as indicated by the above-mentioned results in Mendelian heredity (which indicated that there were probably more separable genes than chromosomes). And in particular the demonstration by Morgan in 1911 of the exchange of sex-linked genes in Drosophila, and Morgan's novel application of Janssen's "chiasmatype" hypothesis of 1909 to explain the fact that these genes showed different degrees of linkage, were from the first regarded by Wilson as most significant. They did much to strengthen him in his conclusions, while, on the other hand, he on his side is to be credited directly and indirectly, as will appear below, with a considerable share in the development of the *Drosophila* work during the immediately following, most formative stages of its existence.

In this period Wilson's conception of the nature of the hereditary material and its relation to the characters of the organism came much closer to the modern one than did that of most Mendelians. This was largely because of his better grasp of three things: (1) the complex physiological relationships of the parts of the organism with one another (as had been brought out long before in his "General Biology"), (2) the mutual reactions of parts with one another during development, as illustrated in many of his and other workers' experiments with eggs and embryos, (3) the nature of the material basis of

heredity, as indicated by the studies on chromosomes and chromatin which now formed his chief interest. Thus he saw beyond the narrow morphological range of the unit-character geneticists and, in an address given in 1911 and again in a paper of 1912 (Study VIII), in nearly identical wording, he gave them the following much-needed admonition:

> . . . Every character is produced as a reaction of the germ considered as a whole or unit-system. Characters are ''borne'' (if the expression is permissible at all) by this system as a whole; and the ''unit-factors'' . . . need be considered only as specific, differential factors of ontogenetic reaction in a complex organic system. Many ''unit-characters'' are known to depend upon a number of such unit-factors, in some cases probably upon a large number; and they may be definitely altered this way or that by varying the particular combinations of these factors. But any unit-factor produces its characteristic effect only in so far as it forms a part of a more general apparatus of ontogenetic reaction constituted directly or indirectly by the organism as a whole.

At other points in the same two articles Wilson entered into a discussion of the composition of the hereditary material which, like the discussion above, served as forerunner of our present view-point. We may note especially the following:

> Kossel makes the pregnant remark that every peculiarity of the species and every occurrence affecting the individual may be indicated by special combinations of protein ''Bausteine.'' The facts lead us to seek for such compounds (substances) in the chromatin or the chromosomes. It can hardly be said that even a beginning has been made in the chemical investigation of the distribution of the chromatin-substances within the nucleus. Cytologically, however, a long series of the most significant facts have been made known in respect to their groupings and modes of distribution . . . and the fact is now more than ever evident that they run parallel to the factors of determination and heredity. . . . It is difficult to see what meaning such processes [the preparations for ordinary mitosis and for the meiotic divisions] can have if they do not involve a linear alignment of different substances which are thus brought into a particular disposition for ensuing processes of division (Roux) or of paired association (Strasburger).

For Wilson, the interest in these matters lay not only in their bearing on the nature of the existing organism and its ontogeny, but also, as previously mentioned, on the related problem of the way in which it has evolved. He realized that the genetic mechanism above discussed

left little room for Darwin's "pangenesis" or other hypotheses deriving the germ plasm from the soma, and made it necessary to look for other causes of germinal variation. Finding no basis for *"entelleche's"* or other fairy tales, he nevertheless—unlike some of his contemporaries among experimental biologists—did not seek to evade the truth that the most amazing thing about life is its complexity of adaptation to self-perpetuation. In this sense, then, as he stated in 1907, Brooks's old epigram is true, that "the essence of life is not protoplasm but purpose." To increasingly explain this by natural processes, in the light of knowledge concerning the nature of the germ plasm, was in Wilson's view one of the greatest objectives of biological work.

In discussions of this problem, he repeatedly returned to consider the theory of natural selection. Thus in 1907 he said: "Evolution by natural selection resolves itself into a series of lucky accidents. . . . For natural selection, pure and simple, the fit is that which happens to fit. I, for one, am unable to find a flaw in this conception of the fit; and perhaps we may be forced to accept it as sufficient. But I believe that naturalists do not yet rest content with it." And in 1915, after recounting the shortcomings of Darwin's views, including Darwin's failure to distinguish between heritable and non-heritable variations, Wilson continued:

We should, no doubt, make a larger allowance for the role of single "lucky accidents" than did many of the earlier evolutionists. And yet, as far as the essence of the principle is concerned, I am bound to make confession of my doubts whether any existing discussion of this problem affords more food for reflection, even today, than that contained in the sixth and seventh chapters of the "Origin of Species" and elsewhere in the works of Darwin . . . we have made it the mode to minimize Darwin's theory . . . but . . . we should take heed how we underestimate the one really simple and intelligible explanation of organic adaptation, inadequate though it may now seem, that has thus far been placed in our hands. . . . While science viewed at close range seems always to grow more complex, a wider vision shows that her signal discoveries are often singularly simple.

In 1930, however, Wilson was "not yet quite ready to admit that higgledy-piggledy can provide an adequate ex-

planation of organic adaptations.'' It is probable that
most geneticists to-day would go considerably farther in
their incorporation of natural selection into their concep-
tion of evolution. Probably they would whole-heartedly
accept the application of the fundamental principle in-
volved to the mutational changes and recombinations of
genetically continuing and multiplying chromosomes and
chromosome parts, and would admit that in this form
natural selection constitutes an integral part—in fact, in
a sense the crux—of the explanation of the adaptiveness
of all living things. But the views of Wilson on this sub-
ject, cautious though they were, were much in advance of
those of the great majority of his contemporaries among
the Mendelians, and, when compared with these, are
clearly seen to lead in the direction of the genetic stand-
point of to-day.

The excitement of the advances in chromosome theory
made by Wilson in 1905–1910 communicated itself
through the department of zoology at Columbia. This
helps to explain why it was that most of the first batch of
youngsters who became *Drosophila* workers with Morgan
had been undergraduates in Columbia College in the latter
part of this period and that others came through by the
same route soon afterwards. Lured on by their first
course in biology, where they were moulded by Sedgwick
and Wilson's text and by the teaching of Calkins and Mc-
Gregor, both former students of Wilson's, some of them
had the privilege of taking in their sophomore year Wil-
son's thrilling one-semester course on heredity and the
chromosomes, variation and evolution. In this the text
chosen by Wilson was Lock's extraordinary book of 1906
—too far ''ahead of its time'' to be sufficiently remem-
bered now—which, with less caution and fewer qualifica-
tions than employed by Wilson himself, advocated the
sufficiency of Mendelism, multiple factors, the chromo-
some theory (including exchange of linearly arranged
genes during parasynapsis, after de Vries) and the nat-
ural selection of mutations, as the basis of all heredity and

evolution. Wilson's superb course on cytology, with its unequalled laboratory training and demonstrations, was usually taken by them in their third or fourth year after entering as freshmen. After this stimulating and thoroughly systematic preparation their embarkation upon the adventure of the fascinating new work on chromosomal heredity in *Drosophila* that had just been opened up by Morgan (1910 and 1911) was the logical continuation, now grown more specific in its direction, of the quest to which they had already become dedicated, calling for the ways of thinking, the knowledge and to some extent even the technique acquired during their previous years of training. And the striking similarity in the attitude of all of them towards the new problems was in no small measure a reflection of the degree to which this common training had been driven home. Thus it is likely that only these *Drosophila* workers, of the earlier years, fully realize to what an extent modern genetics traces its descent through Wilson.

Wilson was himself captivated by the *Drosophila* work —an attitude which he transmitted to his students—and he kept in close touch with it. When a discovery was made in the work he was as pleased as if it were his own. He also appeared from the first as an advocate of the work before other biologists (*e.g.*, in Studies VII and VIII), and he was active in incorporating its results into his system of concepts. Already in April, 1913, in a lecture at the University of Pennsylvania, after restating the evidence from Boveri's work on marine eggs and from his own on sex-determination—which "has definitely established the fact that the chromosomes are causal agents in heredity"—and after presenting the chromosomal interpretation of Mendelism and linkage, he went on to discuss the "attempt . . . made by Sturtevant to calculate from the observed results the degree and character of the twisting of the chromosomes and the relative position of the different specific elements within them." "This," he said, "admittedly is a bold venture

into a highly hypothetical region. Its justification is the pragmatic one that it 'works.' The hypothesis gives us the only intelligible explanation that has yet been of- fered . . . it is just by such venturesome advances that new possibilities of discovery are opened.''

In his Croonian Lecture before the Royal Society, in 1914, he further stressed this evidence for crossing over as well as that, obtained in the meantime, of the corre- spondence between the numbers and sizes of the linkage groups and of the chromosomes in *Drosophila*. In sum- ming up, he declared that ''the conclusion has become ir- resistible that the chromosomes are the bearers of the 'factors' or 'gens.' '' At the same time, elegant chro- mosome studies of other species of *Drosophila* were being made by Metz, working primarily as a student of Wil- son's, which not only gave instructive examples of the types of variation of the chromosome configuration from species to species but, through comparison with the breed- ing results, obtained later, laid the way for a convincing extension of the above cytogenetic parallelism between chromosomes and linkage groups to several other species.

Wilson carefully examined the cytological evidence for crossing over then extant, but saw that it was not yet of a sufficiently critical nature. In 1920, in the course of a critique of the chiasmatype theory, he stated:

I am not able to escape the conviction that somewhere in the course of meiosis some such process must take place as is postulated by Janssens and by Morgan and his co-workers, though I must admit that this opinion rests less on cytological evidence than on genetic. . . . The truth is that for the time being genetic development of the chromosome-theory has far outrun the cytological. We are in no position to predict when the plodding prog- ress of cytology may be able to close the gap: nevertheless we have every reason to hope that the physical mechanism of the recombination-phenomena may in the end prove to be accessible to decisive cytological demonstration.

This expectation has now in some considerable measure been realized, but only during the past decade. In the meantime, however, there were other important ques- tions of cell theory having a bearing on genetics which called for investigation, and in the attack on which micro-

scopic observation still remained obviously the superior, if not the sole available technique. It was mainly to such questions that Wilson devoted his attention in the cytological researches of his later years.

ESTABLISHING THE UNIQUENESS OF THE CHROMOSOMAL ROLE

Wilson's later studies on the organization of the egg have already been mentioned. The other questions with which the researches of his later years were concerned might all be considered variants of the more general problem of to what degree other constituents of the cell than the chromatin may serve as the material basis of heredity. This problem was not one to be taken lightly, for in the case of the chloroplastids definite proof had long before been adduced not merely of their genetic continuity in a general sense but also of the property, which they share with chromosomal genes, of self-perpetuation (involving reproduction) of characteristics differentiating them amongst each other. Since this was true of chloroplastids, might it not, as some authors claimed, be true of chondriosomes, which according to one school of investigators are identical with or give rise to plastids, and might it not be true of other "formed" or "unformed" constituents of the cell?

Wilson kept this question separate from the claim that had sometimes been put forward that the cytoplasm must be as important or more important in heredity than the nucleus in the determination of the fundamental features of early development. It had been argued that this was proved by the observations that had shown the importance, for development, of the pattern of organization of the egg, and by the reciprocal hybridization experiments that had shown the predominant maternal determination of early embryonic stages. In opposition to this argument, Wilson pointed out on various occasions (*e.g.*, in "The Cell," 1925) that the organization of the egg had been proved to be "epigenetically" produced, and that

there had in consequence been every opportunity for the
, chromatin at a preceding stage to affect or control the
egg's cytoplasmic organization. "The whole force of the
evidence," said Wilson in 1925 (and he had expressed
himself similarly much earlier), "drives us to the con-
clusion *that the chromosomes are as much concerned in
the determination of the so-called 'pre-formed' or cyto-
plasmic characters as in any others*" (italics his). And,
as he noted, there are in fact various known illustrations
of such determination. Similarly, Wilson looked askance
at the claim (by Loeb, Jenkinson and others) that the
more ancient characteristics, differentiating the larger
groups, were less likely to be chromosomal than the minor
differences between individuals or races ("The Cell,"
1925, p. 1015).

It is true that Wilson had stated, as early as 1906, "I
no longer hold the view that the nucleus can be considered
as the actual formative center of the cell," but he quali-
fied this by adding, "it still seems to me very probable
that the formative processes are directly or indirectly
under its control, as has been advocated by many stu-
dents of cell-physiology." At times he even went so far
as to say that the chromosomes are probably not "central
governing or controlling factors in the cell" and that
"the chondriosomes are very likely connected with hered-
ity" (1913). But despite these reservations he consis-
tently recognized the at least preponderant role of the
chromatin. The question then resolved itself into this:
May not cytoplasmic constituents have some share in
heredity, and, if so, how much and what kind of a share?
As usual, he did not rest content with answers to such a
question unless they were based fairly directly on a firm
foundation of material evidence.

Wilson therefore undertook, in part with Pollister, to
make a detailed study (1916, 1925, 1931, 1937) of the his-
tory of both the chondriosomes, the Golgi bodies and the
neutral-red bodies ("vacuome") in the spermatogenesis
of scorpions, a group which he had found to show espe-

cially clear and interesting figures of these structures. During the same period, his students and collaborators Bowen, Plough, Pollister and Johnson studied these cytoplasmic bodies in insects. In the scorpions a striking finding of Wilson's was that in the genus *Centrurus* (1916) and in the related *Centruroides* (1937) the chondriosome material becomes condensed before the meiotic divisions into the form of a single ring, which then undergoes "an accurate process of division in the course of which the chondriosome material is precisely divided between the daughter cells." However, in all the other genera of scorpions examined the chondriosome material existed in these stages in the form of a considerable number (*e.g.*, 24) of separate hollow spheroids, which did not divide individually but, becoming scattered irregularly about the spindle, were apportioned with only approximate equality of numbers (5 to 7) among the four daughter cells. Thus the division of the ring in the Centrurids was inferred to represent "a special case, presumably derived from a process of random segregation, as occurs in animals generally."

As for the Golgi bodies, although they underwent remarkable transformations and regroupings at different stages, they were found, even in the Centrurids, to be "distributed to the daughter cells separately, by an operation which offers every aspect of a simple bipolar segregation" of the group, *i.e.*, there was no separation of a half of each Golgi body to each daughter cell. In spermatozoon formation, moreover, although a part of these bodies came to form the acrosome, the main bulk was "cast out in the slough." The neutral-red bodies also became separated in a passive, inexact way, and they underwent complete elimination from the spermatids. Although the conclusion was unavoidable that both the chondriosomes and Golgi-bodies, at least, were real and functional constituents of the normal cell, and that they were continuing structures which somehow became increased in size and underwent fragmentation (not neces-

sarily at or near the time of cell division), so that their numbers were maintained, nevertheless the method of their distribution provided evidence against the assumption of inherent genetic differences between them. They ''can have at most'' concluded Wilson (1937), ''only a very low type of individuality . . . the only actively and regularly dividing elements in these cells are the chromosomes and (presumably) the centrioles. This . . . may be set down to the credit of the chromosome theory of heredity.''

RESYNTHESIS

Even before the rise of the *Drosophila* work a complete rewriting of ''The Cell'' had become imperative, in which the newer findings should be gathered together and organized and the older ones set forth anew from the point of view of the at last definitely crystallized chromosome theory of heredity. So great, however, was the range of time and subjects involved, and so much had observations, experiments and suggestions multiplied, that this was now a Herculean task. It is very improbable that anyone else than Wilson, even with Wilson's earlier editions to build upon, could have accomplished it. In fact, new findings seemed for a time, during the twenty years of the last rewriting, to be accumulating faster than it was possible even for Wilson to incorporate them into a unified account, unless this were to be of far more restricted nature, in relation to the extant body of research, than the earlier editions had been.

To add to these difficulties, Wilson's health, beginning about 1920, seriously deteriorated, causing him to be handicapped thereafter by crippling rheumatism and sometimes by attacks of dizziness. (In this connection it should be pointed out that this illness was in no way—as has elsewhere been asserted—an after-effect of the shipwreck which members of the Columbia department of zoology had experienced off Alaska in 1900: no one had suffered physically in this, and Wilson's health before 1920 had on the whole been good.) Wilson bore these

trials with a remarkable appearance of buoyancy, however, and from then on devoted a major portion of his time to his work on "The Cell."

At last, in 1925, when Wilson was sixty-nine, the completed volume, of over 1,200 pages, three times as large as the second edition, was given to the world. It was indeed as comprehensive as the previous treatments, even in relation to the mass of work that had been published in the meantime. In it virtually the whole of cytology, from the time of its birth more than a half a century before, stood integrated. It was realized by the members of the National Academy of Sciences that, in bestowing the Elliott Medal upon Wilson in 1928 in recognition of this great work, they were maintaining their own honor rather than adding to his. The book was given similar recognition by the Linnean Society.

The main points of Wilson's book will be evident from all that has gone before; a re-examination of its details here is manifestly impracticable. Moreover, it is relatively so recent, and has been so widely welcomed into every-day use by workers in the various fields covered, that a review at this time would be a supererogation. The fact that, during the later years of its preparation, the main concepts of cytology had for the time being reached a stage of comparative stability adds to its indispensability for the consideration of all researches done prior to its date of publication, inasmuch as they form, in themselves, a more or less self-contained system. With this work, if not before, Wilson has rightly been acknowledged to take a prominent place among the great "encyclopedists," if by this term we may denote those who not merely collect knowledge on a prodigious scale but also reinterpret and organize it, translate it into lucid language and make it really usable for investigators as well as for scholars.

It may here be pointed out that after the publication of Wilson's third edition of "The Cell" cytology took another sudden spurt forward in the development of its

underlying concepts. These did not contradict those which had gone before but in a sense went beneath them. Thus, in this most recent period, the significance of the property of paired association of the chromatin material, with its corollary, crossing over, has been shown, through Darlington's work, to go much further in the explanation of genetic phenomena than had previously been realized; at the same time, a unified theory of chromosome structure and structural change, built on the chromonema theory, has been built up. These advances represent the legitimate extension of the work set forth in Wilson's "Cell," proceeding in the same general direction; they connect directly with the framework of generalizations there expounded and do much to further substantiate as well as elucidate them.

Wilson's Attitudes and His Influence

The emergence of these newer concepts serves to illustrate one of the major theses in Wilson's general view of science. For no one, in word or deed, was a more convinced advocate than he of the doctrine that the truths of men are relative. As he said in his lecture "Biology," given in 1907: "Each forward step on the highway of discovery will bring to view a new horizon of regions still unknown." And in 1913, in "Heredity and Microscopical Research": "The explanation of any phenomenon only uncovers new phenomena behind it that still demand explanation, in endless succession; and such is the essential characteristic of scientific progress." In 1915, in "Science and Liberal Education," he expanded upon this theme as follows:

The fundamental concepts of science are in no better case than her weights and measures. They have no finality. They are but a means of advancing knowledge; they move as science moves. . . . No particular law of nature . . . will ever be more than approximate or probable, nor can we state it completely. . . . The profound significance of what we call natural laws lies in the fact that they tersely sum up our experience of the world at any given moment; and, above all, they endow us with a gift of prophecy that leads us on to new advances. Just here we are in sight of what is most vital, characteristic and hopeful in the spirit of modern science . . . this ideal [that of science] is, in a single word, *progress.*

In various articles Wilson has given us, in vivid and poetic language, his conception of the psychology of the scientist, and in this portrayal much in his own character stands revealed. Thus, in his article of 1915, above quoted from, he also said (italics his): "Science . . . should adjust our vision to the larger meanings in the material world. And by this I mean to say that *science should develop—and it should discipline—the constructive imagination.*" This, he maintains, "is the best gift of science to our intellectual life." Regarding the role of the imagination in science itself, he continued:

> In every field the great discoverers have been seers, men of imaginative vision, carried onwards by swift intuition that runs far in advance of solid fact or rigorous logic and ranges freely to and fro in undiscovered realms beyond them. And this is a true creative process, one that is singularly like what we call the inspiration of the painter or the poet. It often thrills us in the same way. . . . At every point the material world overflows with half-revealed meanings about which science is forever weaving her imaginative fabrics; and at their best these have all the freedom, boldness and beauty of true works of art.

But Wilson was well aware that, unlike art, science cannot be satisfied with any construction that achieves only an inner harmony, no matter how appealing. For it has, alas for many a brilliant mental construction, to match its own creations against the phenomena of the outer world, and for this reason the discipline of its imagination must be the greater. "One conspicuous trait, indeed," said Wilson in the same article, "distinguishes the man of science—his incorrigible, almost automatic insistence upon verification. For no one better knows that the children of his imagination will live only in so far as they take on the living flesh and blood of reality in the appeal to nature. Not many of them survive the ordeal; yet they are the pioneers of progress, and the real conquerors of the world."

But for the scientist, said Wilson in 1909, in "Teaching and Research in the Natural Sciences," it is not even enough to

combine mental grasp, constructive imagination and natural aptitude, with an acquired mastery of his subject. . . . These will not avail if there be not

added an impulse that grows from a lively interest in the phenomena of nature and a spirit that demands to know the truth. The great theories of science possess a very high degree of fascination, her sweeping generaliza- tions make a powerful appeal to the imagination. But they have been built up little by little through the hard and plodding study of concrete facts; it is only through such study that science moves forwards. Those who have not a first-hand acquaintance with the actual methods of research have no conception of the amount of "dead work" that it demands, of the concen- trated patience that must be expended on purely technical processes, on the painstaking and conscientious accumulation of data that may long seem to give no tangible result. The investigator must prove all things, and he must have an insight and imagination trained to hold fast to that which is good. The motive power that carries him through his tasks is something akin to the artistic impulse, though it finds so different a mode of expression. It is in the best and largest sense the love of nature. It is a spontaneous interest in the world of natural phenomena that will spare no labor to find out the least as well as the greatest things and finds its best reward in the discovery of their orderly relations. Let the student ask himself in what way he feels drawn to the study of science and what will be his attitude towards his daily work. Is his imagination stirred only by the grand theories of science or the hope of making great discoveries? Science calls for some- thing more direct and substantial than this. Her first demand is to know what things are and what they do. Her first interest is in concrete phe- nomena; in the physical features of the earth, in the substances of which things are made, in animals and plants; in the actions and interactions of things, in the relations of cause and effect among them. One who is con- scious of such an interest, who can find satisfaction in the truth-seeking study of natural phenomena for their own sake as well as for the larger meanings that underlie them, has at least one of the best grounds on which to base the hope of success, and he will find scientific work worth while. Such an inter- est will broaden and deepen as he goes forwards. It gives the impulse that has led to all the great discoveries and all the great generalizations of science.

The above should be taken in connection with another passage, written in 1900, in which Wilson expounds upon his conception of the primary operations of science as follows:

No one, I trust, will understand me to advocate the indiscriminate accumu- lation of facts—for this is not method, but the absence of method. The essence of science is not the accumulation of knowledge, but its organization. Observation and experiment give us our materials, but it is the comparison and correlation of these materials that first build them into the fabric of science.

It should be noted that when Wilson used the word "progress" in the passages previously quoted he was concerned with actual human progress, in the fullest sense of the term, for his nature was a broad and under-

standing one and his sympathies were with his fellow men. He was not one of that group of chemically-pure scientists or art-for-art's-sake-alone artists who feel defiled at human contact or at any value their work may have in raising the lot of mankind. This was shown not only in his attitude towards men and things in general, but also in specific utterances. Thus, in 1909, after noting with approval the opportunities for scientific research that were rapidly being opened up in government service and in industry, he made the comment:

The significance of this is not lessened by the fact that many of these activities involve the application of scientific discovery to practical or commercial ends, that they graduate almost insensibly into work of a purely technical or industrial character. The boundary between ''applied'' and ''pure'' science has almost vanished. The day is past when the investigator could hold himself aloof from the applications of his science to practical affairs. He whose life is given wholly or in part to new applications of knowledge to human welfare may be as truly an investigator, and may serve mankind as well, as he who seeks only to extend the boundaries of knowledge. The aim of a Lister or a Pasteur is not less lofty than that of a Laplace or a Lyell.

As late as 1930, in commenting on the past quarter century's progress in lines of biology dealing with the nature of the finished organism, he said:

What first comes to mind is its splendid service to human life . . . man has built no temples more splendid than our medical schools, hospitals and institutions for medical research. . . . The new discoveries in heredity are steadily at work for the improvement of our cultivated plants and animals, while the agricultural schools and experiment stations, now at work in every state in the Union, may well merit a place on the same role of honor. . . . Our knowledge of the chemical basis . . . has at least threatened to invade the field of sociology. . . . It is in this direction that biology may justly be said to have some legitimate concern with those broader human activities, such as history, literature or ethics, which at first sight seem so remote from contact with our science.

As either scientist or artist, then, Wilson belongs in the class of those moderns, as well as those of remoter ages, who conceived of their work as an integral part of human life as a whole, and who, in addition to their direct engrossment and joy in the activity itself, derived inspiration from their consciousness of its relation to that life and of its ultimate effects thereon.

The various quotations given above should make clear much of the motivation back of Wilson's own achievements. It is apparent that, in the depth of his nature, the artist and the scientist were one. Thus to a considerable extent the same gifts and feelings which enabled him to be a great cytologist led him, on the other hand, to his deep appreciation of music and his remarkable facility in musical expression. His wide knowledge and understanding of chamber music and of its literature and his power of musical analysis were second only to his mastery of the field of cytology, while his ability as a cellist was such as to have led to his being rated by a famous musician as the foremost non-professional player in New York. For many years in fact he spent much of his leisure playing in a quartet that did include distinguished professionals. This afforded him an invigorating outlet for energies otherwise kept strictly confined. Whereas in his research work, as in his lectures, writings and general conduct he held his inner warmth of nature restrained and harnessed by his rigorous discipline to such an extent that it served as an underlying motive which seldom could break through completely to the surface, in his music this rich emotional drive had freer play and achieved a degree of release that science could give it only at rarer moments. Perhaps the opportunity for its exercise provided in this way enabled Wilson the better to hold his internal fire so firmly in rein when in the performance of his scientific labors.

It was not only in Wilson's love of the immediate objects which he contemplated and in his thrill at the symphonies of scientific law wherein they at last became reorganized and woven together that his artist's temperament played a vital role in his work. It was equally operative in his pronounced spirit of craftsmanship. In his music, this expressed itself in his being so accomplished a cellist, in his outdoor recreation, in his being a master sailor, and in his work it was evident in the felicitous manipulation of his superfine materials, in the beauty of

his microscope preparations and in the perfection of his drawings. No less was this spirit active in the sphere of verbal expression, in the communication of his findings and conceptions to fellow scientists, students and the public, whether through articles, books, lectures or even through ordinary conversation. Thus, each of the lectures in Wilson's various courses—introductory biology, evolution and heredity, cytology, cellular embryology and general zoology—was an artistic as well as scientific creation, a finished product of model composition; thorough in its inclusion of material, yet lucid, elegant and full of charm, and having all its parts systematically arranged and subordinated to the whole structure.

At the same time, Wilson's lectures and writings seldom displayed the literary efflorescence that his first impulse would have given them. It was his practice ruthlessly to trim them down in order the better to fit them into complete subservience to the material dealt with. This made them ideally suited to the needs of the scientist and the student, a circumstance which played an important part in the wide influence on science which Wilson came to have. The same exceptional discipline, sometimes carried to the degree of what some object to as "leaning over backward," usually dominated Wilson's criticisms of others, his rare utterances on hotly contested topics of philosophy or general affairs, his scientific theorizings, and even his outward demeanor. Thus this lover of nature and this adventurer in high places, with his soul of an artist, appeared as "a quiet gentleman."[1] This paradox was a fortunate one, for it was conducive to his being granted many mundane marks of recognition which, showered upon him even by those who were not in a position to appreciate his deeper meanings, helped to create conditions under which his work could prosper and could reach a wider circle of those who would really appreciate and make use of it.

[1] The term used in a very valuable obituary in the *New York Herald-Tribune*, March 4, 1939.

Those of his associates and students who were not so patient as Wilson himself would, however, thrill at an occasional more direct glimpse of the hidden part of his inner nature, shining through in the form—for instance —of sly humor, biting criticism or far-flung aspiration. It was refreshing, for example, to discover that in his classical paper on earthworm embryology, in 1890, he had delivered the following well-deserved rebuke to an unfair critic whom we need not name: his "paper, as a matter of course, exhibits in some degree the well-known and characteristic skill of its author in belittling the work of other investigators, but I observe with interest many signs of progress." A similar flash was witnessed by the present writer in 1910, when, at the would-be gravest moment in a disgustingly pretentious ceremony, Wilson, sitting across the room on the platform, caught his student's eye and risked a quiet wink. Such rare moments were the more revealing because of Wilson's outstanding mildness, courtesy and dignity in manner, word and deed.

For Wilson's conformity was not ordinariness, and he reserved his spirits for more worth-while ends, hidden to the passerby. Where others saw only dust or monotony, he might discern harmonies. When in his youth he had read Aristotle and Hume, it had been an expression of his ebullience, not of the desiccation that the public associates with ancient volumes. In his later years, this "quiet gentleman" was not the type of colorless professor that some who met him casually might have assumed. At sixty, Wilson could still delight in Wells's fantasy "The First Men in the Moon," and at 74, in his lecture of 1930 on "Biology," he could not refrain from a sympathetic public reference to the idea of ectogenesis as depicted in Haldane's "Daedalus." At this age and even at 83 there still lived the Wilson who exulted in music and in the colors of Naples, who was fascinated by a beetle and an amphiastral figure, who found exhilaration alike in battling the wind and waves in his boat and
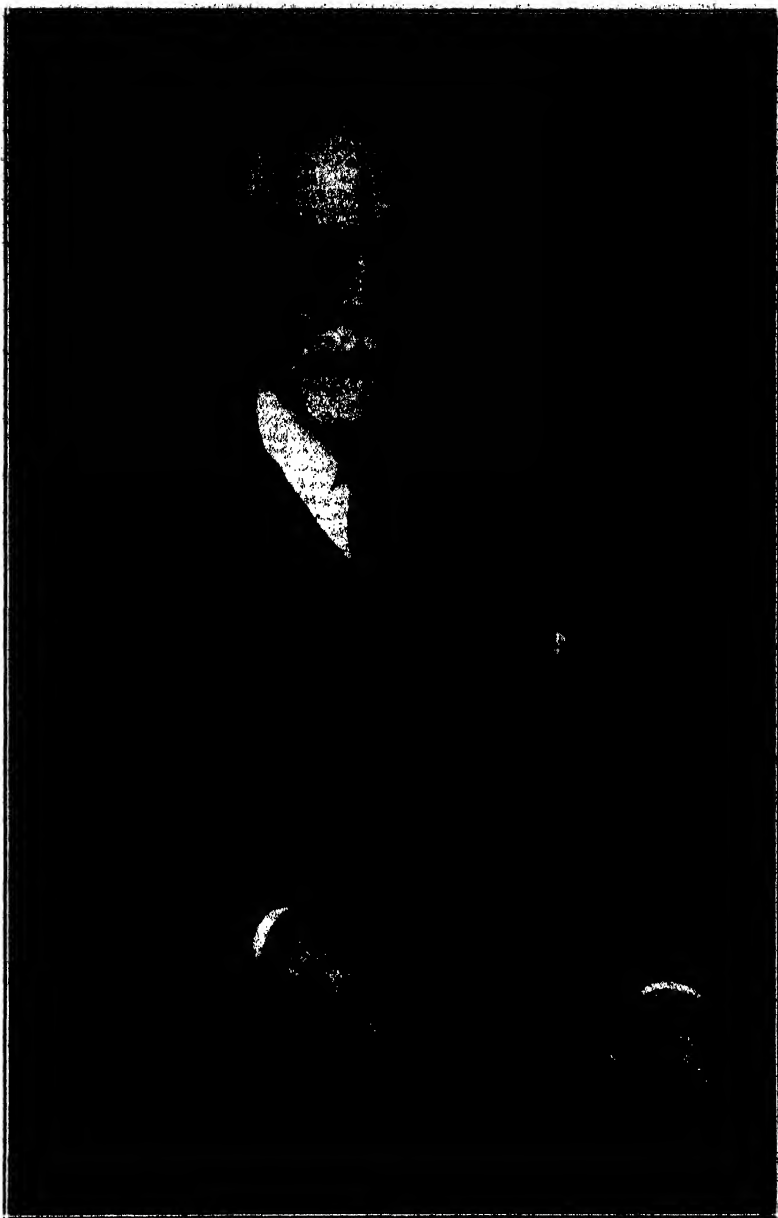
Wilson at eighty-one, in his tenth year as Professor Emeritus at Columbia, but still engaged in research. This year, 1937, marked the appearance of his last paper: that with A. W. Pollister, dealing with the method of transmission and the significance of achromatic bodies.

in helping steer the course through the restless sea of facts and hypotheses of genetics. For Wilson, in his later years, was an example of that rare type of aged persons whom it is hard for us to believe to be really old, whose minds have stayed plastic and inquiring, whose sympathies are active, and who are not increasingly engrossed in ever diminishing selves. And through it all he was of those who retain the humility of strength.

It is not surprising that he whose nature it was to love and understand the animals and even the insensate things that came within his care, and who responded so actively to the deepest feelings of great artists, should also have feelings of warmth and sympathy for the people who were under his guidance. This no less than his intellectual preeminence explains the affectionate reverence in which his students and colleagues held him, and the extent of his influence over their attitudes and concepts. His students meant very much to him, and he took his responsibilities in connection with them very seriously. By a natural reciprocation, he filled a large place in their own lives. And after they had passed out of his hands, they found that even more important to them as scientists than the facts he had taught them were the attitudes which he had helped to imbue—especially the spirit of objectivity, of criticism, of seeking out truth even in error, the demand for verification, the striving for organization, the ideal of progress.

The harmoniousness of his human relationships, and their deep meaning to him, are likewise evident in his sincere and intimate friendships, both with comrades in this country and with those whom he had met abroad, among the latter especially Anton Dohrn and Theodor Boveri. These friendships were marked by that ideal intimacy of communion throughout varied spheres of living whereby the lives of both participants experience a thoroughgoing mutual enhancement. But most brightly of all Wilson's nature shone in the profound happiness of his married life. He was married to Anne Maynard Kidder in

1903, shortly before the most creative period of his scientific work. His life with his wife, and with their daughter, Nancy—now Mrs. John Lobb,—was an idyl such as is seldom met with.

We shall not speak in detail here of the various administrative posts which Wilson filled with distinction. In all these he strove to foster that spirit of science in which he had his being. Sometimes, however, he doubted whether, with the multiplication of administrative and teaching activities in a modern university, it "could maintain an atmosphere in which scientific research and scholarship can freely breathe," and he warned that "the leadership of the universities in intellectual progress will depend on the reply" made to that question.

Neither shall we recount here the numerous signal honors which Wilson received. They are to be found listed in various directories. They serve as indications of the breadth and the strength of the influence he exerted, though to assess the value of that influence one must know the history of cellular biology and genetics.

It is indeed fortunate, not merely for Americans but for all mankind, that Wilson should have succeeded in transferring such a vigorous scion of the cell-science of Germany and the Low Countries to this section of the English-speaking community in those years, near the turn of the century, when its growth over there was most flourishing. Equally important is the later impetus he gave it, which played so direct and crucial a part in the incorporation of cytology into genetics as its physical basis, and of which all modern work on the chromosome theory of heredity is so largely an outgrowth. In the other main section of the English-speaking community also, as well as in the U.S.S.R., Scandinavia, Japan and elsewhere, this work has for some time past been taken over, largely as a result of the success which the point of view fostered by Wilson had in this country to begin with. In consequence the work has been carried much further forward, especially in the decade now closing, in an ever greater and more coordinated world effort.

The fumes of war, spreading out from the heart of the region where the cell theory had its first rise, are now grievously choking this growth in an ever wider circle. But science must grow or retrogress. Thus the duty now falls back with increasing urgency upon us who remain, of continuing to nurture this precious and sensitive growth in our own midst, and of assisting it as best we can wherever else it may still have a chance to survive. This surely is what Wilson would have counselled. It represents the continuance to-day of the work in which he lived. And in this work and in its further extensions in times to come, so long as science lasts, his spirit will lead us forward.

REVIEWS AND COMMENTS

IN this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution.

REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

Systematics and the Origin of Species. From the Viewpoint of a Zoologist. By ERNST MAYR. New York: Columbia University Press, 1942: i–xiv, 1–334, figs. 1–29. $4.00.

THE emergent science of speciation is notably furthered by the publication of this book—the most comprehensive treatment of the subject in the English language. The new systematics is advanced not only as a special field of inquiry but also as an integrated element in general biology. A broad groundwork of sound generalization is laid for the construction of a modern philosophy of speciation.

Of the many contributions in this volume perhaps the most helpful to American students of speciation will be the data and interpretations of European systematists— of specialists not only in birds but also in many other groups, particularly insects. Mayr's familiarity with the far-flung systematic literature is amazing.

The treatment is perhaps least adequate for the aquatic vertebrates. The great richness of data derived from racial investigations in fishes is little exploited. The "ecological rules" of the mammalogist and the ornithologist are discussed at some length, but those of the ichthyologist are inadequately presented. Little credence is given to the very strong evidence that speciation of fishes has been explosive where, as in newly formed lakes, an unsaturated set of habitats has suddenly been provided.

It is only natural that the author should stress the data and the viewpoint of systematic ornithology, and in some

ways his book has thereby been strengthened. Certain aspects of speciation can be most advantageously developed from an ornithological approach. As the reader is reminded (almost too often), birds as a class are the most completely described and named, the most extensively studied, of any group of animals. For this reason ornithology provides in general the most ample material on which to base tabular analyses of different types of speciation. Following Rensch's lead Mayr is able to summarize relatively complete arrays of case data, to contrast the insular and the continental types of differentiation; to postulate relations between dispersal and subspeciation, as on archipelagos; to determine how, where and.to what degree geographic forms intergrade; and to illustrate other speciational trends. Mayr's own studies along these lines, on the birds of the now battle-torn southwestern Pacific, prove to be particularly illuminating.

In other ways, however, the treatise seems to have been weakened by the ornithocentric approach. Phenomena, interpretations and methods of study that are better illustrated by groups other than birds receive relatively little emphasis. At times the impression is given that the author is forcing the evidence derived from other groups into the pattern of speciational interpretations which he has built up from the bird data. Thus the evidence for ecological, temporal and behavioral isolation is (in my opinion) too strongly discounted, in favor of the view that almost all speciation, in other groups as well as in birds, is attributable to geographical isolation. Geographical representation as a practical species criterion (though admittedly a very important one) seems to have been overdone. There is perhaps an overstress too on the contrast between sympatric and allopatric speciation. Incidentally the etymologically hybrid terms sympatric (not isolated) and allopatric (isolated), referring to kinds as well as to speciational types, might better be written "compatric" and "alipatric."

The slight attention paid by Mayr to experimental researches (perhaps because such studies have received little emphasis in systematic ornithology) constitutes a measure of backsliding in a forward-stepping movement. Experimental work, when occasionally and briefly referred to, is relegated to genetics—as though experiment were the prerogative or characteristic of certain branches of biology, rather than a method of research available in all divisions of science. The increased application of the experimental method in systematics is surely one of the reasons why systematics and other branches of zoology are coming to a rapprochement, which in other ways Mayr has done very much to further.

The statistical approach to speciational problems though favored in principle is likewise slighted (perhaps because exact quantitative methods of analysis have been less used in ornithology than in some other branches of systematic zoology).

The line of thought that is most persistently put forth in ''Systematics and the Origin of Species'' is that the ''morphological species concept'' must be replaced by a ''biological species concept.'' The two concepts are subjected by Mayr to a rather intense verbal contrast: the morphological criterion is held to be antequated, unnatural, nonfunctional, static, and quite inadequate as a basis for the study of speciation; the biological species concept is said to be modern, natural, functional, dynamic, and brimful of significance for the analysis of evolution.

In only one practical application, however, is any clear difference between the concepts demonstrated. In accrediting specific rather than subspecific status, Mayr relies not on the morphological criterion of amount or kind of difference but rather on the attainment of actual or potential genetic isolation, in nature. The distinction between the ideas does not apply to pairs of forms which come into contact with one another, for on either concept the kinds are then regarded as conspecific if they interbreed and intergrade. It appears that the distinction

applies only to pairs of forms which do not come into contact: the "morphological" systematist then grants specific status if the two kinds are consistently separable, or widely different, whereas the "biological" systematist (of the Mayr school) accords the specific rank when from varied data he infers—ordinarily by necessity guesses—that the isolated forms would not freely interbreed in nature if they were to occur together. But such judgment will ordinarily have to rest on morphological evidence (as Mayr admits): if the differences between the two isolated kinds is of the order of the distinctions between forms which come together but fail to intergrade, then, by analogy, the isolated forms are called species. Yet such morphological criteria are held in disrepute, and regarded as virtually useless because many true subspecies are more distinct in characters than some full species!

Mayr's "biological species concept" ordinarily slams the door on any objective test of the specific distinctness of geographically isolated forms (for which alone it is distinctively applicable). Since his one criterion is that of genetic isolation *in nature*, one can not on his basis test the specific status of any given pair of forms by *experimental* matings. Only by transference experiments, which would very seldom be practicable to conduct or to follow critically, could one determine whether two forms would freely interbreed to produce fertile offspring in nature. This "biological species concept" would therefore force systematic procedure (and speciational considerations based thereon) into the realm of impression and authority. It seems to me that this is not the thing to do, since an outstanding need in systematics is the replacement of an impressionistic and authoritative treatment by an objective one, capable of being verified by research rather than by an agreement in opinion. It is perhaps of some significance, in this connection, that impression and authority have played a particularly strong role in bird systematics.

To define the species as Mayr does solely on the basis of actual or potential genetic isolation in nature, and to base a systematic procedure and a speciational philosophy on this sole criterion, certainly has the pedagogic advantage of simplicity—but is not thereby necessarily justified. On the contrary, the excessive complexity and variety of the speciational process renders such a definition (and theory and practice) inadequate.

The most obvious criterion of the species would seem to be the attainment of complete differentiation. Genetic isolation (actual but not potential, I would say) is one of the main criteria involved in measuring such divergence, but the evidence for genetic isolation often breaks down. As Mayr notes, a mere mutant may be more or less sterile with its parental type, whereas some distinct genera produce fertile offspring, and all grades of interfertility and intersterility may be observed in experimental systematics. Genetic isolation is a usual but not an invariable mark of the specific level. The same can be said of a notable amount of structural difference, or of a great complexity of differential characteristics, or of intersterility in experimental matings as well as in nature, or of sharp differences between types in behavior. I think that all such criteria need be considered, in both theory and practice.

If we accept completeness of differentiation as the test of the attainment of the species level of differentiation, and if we base our interpretations on functional as well as structural characteristics, and regard the species (and its subdivisions) as populations maintaining themselves by their own life ways in their own environments, we arrive at a species concept that is quite as modern, natural, functional and dynamic as is the ''biological species concept'' of the book under review.

Mayr duly emphasizes the idea that the systematic categories (minor races, subspecies, species, genera and higher groups) are populations, and his discussions are replete with indications that these populations are

marked by integrity in time and in space. He does not, however, join with other new systematists in definitely expressing the corollary that the history, consolidation and survival of a population, in other words, its successful mass reaction in the struggle for existence, constitute a systematic criterion that is quite as significant as the genetic potential. As though either ignoring or discarding this concept, Mayr writes of the instantaneous origin of new species through such means as polyploidy—rather than writing of the origin by such methods of the genetic material out of which in time new species might arise, through a long ordeal of survival and integration.

Notices of New Books

The Handling of Chromosomes. By C. D. Darlington and L. F. La Cour. New York: The Macmillan Co., 1942: 1–162, pls. 1–16, figs. 1–7. $2.50.—We have here an excellent *vade mecum* for the preparation of cytological material, clearly and authoritatively written. The methods must be good, if they will yield even occasionally results to compare with those shown in the 16 plates of magnificently reproduced photomicrographs.

Man and the Biological World. J. Speed Rogers, Theodore H. Hubbell and C. Francis Byers. New York. McGraw-Hill Book Co., 1942: i–x, 1–607, figs. 1–180. $3.50.—Textbooks are not often given attention in this review section, but occasionally one appears that is too outstanding to be left unnoticed. The text of the University of Florida biologists, which has now been made generally available, fits into this category. It presents with even balance the whole array of fundamental biological principles. The human aspect is stressed, but without undue emphasis. The frank viewpoint on such problems as evolution and sex is especially commendable. "Man and the Biological World" is not only an excellent text for a year's course in biology; it will also serve well as an elementary biological reference work in schools and home libraries.

Guide to the Literature of the Zoological Sciences. By Roger C. Smith. Minneapolis: Burgess Publishing Co., 1942:

i–vii, 1–128 (offset printing). $2.00.—Students often go through their training in zoology without learning how to find and use the vast literature in this field. Only a few zoology departments offer a course (or seminar) on zoological literature. One of the best of these courses, to judge from the excellent outline in Smith's *Guide*, must be the one given at Kansas State College. "Everything a Young Student Should Know about Zoological Literature" might have been chosen as the title of this book. It tells one about the use of a library, location of titles, dictionaries, encyclopedias, gazetteers, atlases, maps, biographical dictionaries, abstract journals, preparation of bibliographies and of scientific papers, nomenclators, special indexes and species catalogs, general taxonomic works, and many other items.

Introduction to Parasitology. By A. S. PEARSE. Springfield and Baltimore: Charles C. Thomas, 1942: i–viii, 1–357, figs. 1–448. $3.75.—Few biologists of the day can match Pearse in command of information or in power of synthesis. These traits are exhibited in all his books, and stand out sharply in this treatise on general parasitology. Most texts in the field are largely limited to medical parasitology, or deal almost entirely with the main groups of parasites; this one covers parasites in a broad and inclusive way. Many biologists, even some parasitologists, will be surprised to note how widely the phenomenon of parasitism is spread throughout the animal kingdom. Beyond the 8-page introduction, the treatment is entirely systematic, but much information on the life-cycles of parasites and on their ecological and pathological relations is given throughout the book. The well and interestingly written text is fortified by an abundance of fine illustrations.

The Plant Communities of the Welaka Area. With Special Reference to Correlations between Soils and Vegetational Succession. By ALBERT MIDDLETON LAESSLE. University of Florida Publication, Biol. Sci. Ser., 4, 1942: 1–143, pls. 1–14, figs. 1–25, maps 1–3, charts 1–2. $1.50 plus postage.—This is a thorough ecological study of the University of Florida Conservation Reserve. It is an important contribution to plant ecology, and will serve as the basis for other researches being conducted in this area by the active biological staff of the University of Florida.

Science in Progress. Third Series. Edited by GEORGE A. BAITSELL. New Haven, Conn.: Yale University Press, 1942: i–xiv, 1–322, figs. 1–112. $3.00.—The third series of the national lectureships of the Society of the Sigma Xi comprises ten lectures which were presented in 1941 and 1942 by highly competent authorities. Astronomy, physics and engineering are emphasized. Photosynthesis, one of the most complicated and fundamental of the problems of life, is presented very skilfully and interestingly for the general scientific reader by JAMES FRANCK. Medical science and physiology are represented by a chapter on a timely and important subject, "The Mode of Action of Sulfanilamide," prepared by PERRIN H. LONG. Many biologists will turn with keenest interest to V. K. ZWORYKIN's very informative article on "Image Formation of Electrons." The electron micrographs of microorganisms and of a filterable virus are very impressive. The volume maintains the high book-making standards of the Yale University Press.

Handbook of Frogs and Toads. By ANNA ALLEN WRIGHT AND ALBERT HAZEN WRIGHT. Ithaca, N. Y.: Comstock Publishing Co., Handbook of American Natural History, 1 [Ed. 2], 1942: i–xi, 1–286, pls. 1–88, 30 figs. $3.00.—Bountifully illustrated and enthusiastically written, this book has been prepared for and by naturalists. It should be useful too for the many experimental biologists who employ amphibians in their researches. Systematists will criticize the lack of consistency in the nomenclature of typical subspecies: some are designated by the species binomial, thus usurping the name that properly belongs to the whole species complex; others are correctly accorded the tautonymic trinomial.

SHORTER ARTICLES AND DISCUSSION

INDEPENDENT IDENTICAL MUTATIONS TO ALBINISM IN THE SEX CHROMOSOME OF THE FOWL[1]

Following our earlier report of a type of sex-linked, imperfect albinism in the domestic fowl, two other cases of albinism, geo-graphically widely separated, were brought to our attention. Representatives of each were secured for study and appropriate genetic tests have now proven that these mutations are identical with that first studied by us. Because there is good evidence that the three mutations to albinism occurred quite independently, they are of sufficient interest to warrant this brief report. The histories of the three cases follow.

(1) *In New York State: in Barred Plymouth Rocks.* An albinotic chick obtained in 1939 from an Ithaca hatchery developed into a female that showed faint "ghost-barring" in the plumage and the presence of some melanin in the eye. The pupil appeared dull red. In albinotic descendants of this bird, melanin was present in small amounts in the pigment epithelium of the retina, and in the retinal portions of the ciliary body and of the iris. There was none in the choroid, which is pigmented in normal fowls. A more detailed description has been given by Mueller and Hutt (1941). Their genetic studies showed that the condition was caused by a sex-linked, recessive gene which was designated *al*.

In about 8,000 Barred Rock chicks from the flock that yielded this mutation, only the one albinotic chick was found. Any others would have been equally conspicuous and therefore none could have been overlooked. Since (1) any sire heterozygous for the mutation would have carried it in half his germ cells and caused albinism in half his daughters, (2) the average number of chicks per sire in this flock was about 270 and (3) only a single albinotic chick was obtained, it was considered probable that the sire of our first albino had not been heterozygous, but that the mutation had occurred so recently in his germ cells that only a small proportion of his spermatozoa carried the gene, *al*.

(2) *In Massachusetts: in Barred Plymouth Rocks.* Through the kindness of Dr. J. C. Scholes, of the Beacon Milling Company, Cayuga, N. Y., we obtained in 1941 an albinotic Barred

[1] No. 17 in the series, "Genetics of the Fowl," by the senior author.

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Rock chicken that had been brought to the agency of that company in Fall River, Massachusetts. Nothing was known of its history, and apparently only one such chick was found in that locality. It seems improbable that this bird could have been related to the albinotic chick obtained at Ithaca, even though both occurred in Barred Rocks. It developed into a female that showed the same ghost-barring as did our first albino. After it matured, it was mated with an albinotic male that was homozygous for the Ithaca mutation. The results (Table 1) showed clearly that the two mutations were identical, for all the progeny were albinos. If the two mutations had been different, a ratio of 2 normal $\male\male$: 1 normal $\female$: 1 albinotic $\female$ was to be expected.

(3) *In Indiana: in S. C. White Leghorns.* Upon following up reports of pink-eyed chicks among White Leghorns at a hatchery in La Porte, Indiana, it was learned from the proprietor that he had hatched upwards of 80 such chicks in 1941, and that all were females. This indication that a sex-linked mutation was responsible was confirmed by breeding tests at Ithaca with two albinotic Leghorns that survived to maturity from the five that were kindly sent to us as chicks by the owner of the hatchery. When these were mated at different times with two males of the Ithaca stock, one homozygous and the other heterozygous for the gene, *al*, the results were as shown in Table 1.

TABLE 1

TESTS FOR IDENTITY OF THE MUTATIONS FROM THREE STATES

Mating	Progeny				
	Albinos			Normal	
	Males	Females	Not sexed	Males	Females
1. Ithaca ♂, H4998, *al al*, × Mass. albinotic ♀	5	5	5	0	0
2. Ithaca ♂, H4998, *al al*, × Indiana albinotic ♀♀	5	0	13	0	0
3. Ithaca ♂, H10042, *Al al*, × Indiana albinotic ♀♀	7	7		7	11

It is clear, from the data given in Table 1, that the mutation in the Indiana White Leghorns was identical with that in the Barred Rocks at Ithaca. Had it been otherwise, the second mating listed in the table would have yielded only normal males and albinotic females, and the third would have yielded a ratio of 2 normal $\male\male$: 1 normal $\female$: 1 albinotic $\female$. The albinotic males hatched from all three matings could not have been obtained un-

less their albinotic dam carried the same gene, *al*, as did their sires. It follows that all three mutations were identical.

DISCUSSION

For reasons given above, it was considered that the mutation at Ithaca was discovered in the first generation after that in which it occurred. Attempts to trace the Indiana mutation further back failed. The owner of the flock to which the hatchery operator assigned the albinos stated that his White Leghorns were under official supervision in a Record of Performance program, and that any peculiarity such as albinism would certainly have been noticed, either by him or by the inspectors. Because sex-linked mutations in the fowl are bound to appear in the daughters (which are heterogametic) of any heterozygous sire, they are recognizable, if their effects are visible, soon after the mutation occurs. Unlike autosomal mutations, they are not carried along unseen for several generations until chance brings two heterozygous breeders together. It seems probable, therefore, that all three of these mutations occurred within about a year (one generation) of the time when they were discovered. This supports the other evidence that all three mutations occurred independently.

Apart from the fact that the Indiana mutation appeared about 700 miles from that found at Ithaca, the occurrence of these two in two different breeds is evidence that they arose independently, especially because both came from pure-bred flocks used to supply first-class hatcheries.

Only one case of albinism (Warren, 1933) and one of pink-eyed dilution (Warren, 1940) were reported in the fowl, so far as the writers can ascertain, in the first forty years of this century. It is somewhat of a coincidence, therefore, that there should be discovered three separate cases of albinism attributable to independent, but identical, mutations in one locus of the sex chromosome. The coincidence was heightened by the appearance in 1940 of a type of sex-linked albinism in the turkey that differs somewhat from that in the fowl (Hutt and Mueller, 1942). However, it seems probable that the appearance of these four sex-linked mutations in three years must be attributed, not to the action of any cosmic agency that might have accelerated the mutation rate, but merely to the fact that there are now more persons in the field than in earlier days who are able to recognize

items of genetic interest and who know where to send such items for study.

SUMMARY

By breeding tests of albinotic fowls found in New York State, in Massachusetts, and in Indiana, it was shown that all three were genetically identical, and were caused by a mutation in the sex chromosome. In one case, the mutation occurred in White Leghorns; the others were in Barred Plymouth Rocks. Reasons are given for assuming that the three mutations, though genetically identical, arose quite independently.

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EFFECTS OF SINGLE GENES ON THE BEHAVIOR OF DROSOPHILA

A NUMBER of years ago the author became interested in the problem of the practical importance of hereditary variation. What animals (or people) do is presumably more important than their appearance, which has long been known to be largely controlled by genetic factors. Therefore, an attempt was made to find a simple but important behavior trait in an animal already genetically analyzed.

It was assumed that the behavior of insects would follow a simple, unvarying pattern, and the photic response of *Drosophila melanogaster* seemed to present ideal raw material. This reaction, which Carpenter (1905) defined as the fly's tendency to move toward light when mechanically jarred, appears to be highly useful in facilitating its escape from garbage pails and cavities in rotten fruit.

Following suggestions by Dr. H. H. Strandskov, certain Drosophila stocks carried at the University of Chicago were investigated. Preliminary work (Scott, 1937) showed that there were distinct differences between brown and white stocks in both mean and standard deviation of the times for crawling toward a light (brown 14.49 ± .23, 1.46 ± .17 seconds; white 21.23 ± .76, 4.18 ± .54 seconds).

As these stocks had been maintained for some years by transferring a few flies to a new bottle, it was assumed that they were inbred and that the differences were genetic. It was also shown that the differences were mainly caused by an unusual sensitivity to jarring in the brown stock.

This supported the theory that important differences in behavior can be produced by heredity but gave no evidence as to the role of single genes. The next step was to test for possible effects of the two known genes in the stocks.

METHODS

The difficulty in this sort of experiment is to eliminate the possibility that other factors closely linked to the known genes will affect the character. In this case it was attempted to get rid of these extraneous factors by backcrossing. A red-eyed fly was crossed with one from the brown stock and a red-eyed daughter (in which crossing-over could occur) was backcrossed to the brown stock. Her red-eyed daughter was again backcrossed and this was repeated so that in each generation there was an opportunity for some of the extraneous genes brought in by the original red-eyed fly to be lost. The same procedure was used in the white stock.

The reaction of the flies to light was tested by essentially the same method which was used in previous work (Scott, 1937), except that it was possible to keep environmental conditions much more closely controlled. Normal and mutant flies could be raised in the same bottle from the same parent and be tested within a period of two hours.

The apparatus used is shown in Fig. 1, and the primary data obtained with it represent the median times for a group of 19 flies to start and crawl a horizontal distance slightly greater than 18.3 cm, in a beam of light ranging in power from 6–16 foot candles, at a temperature of 24–25° C., and under conditions in which the only vibration came from the flies running into each

FIG. 1.   Apparatus for measuring the reaction of Drosophila to light. On the right, a rack holding a glass tube with removable end sections, an opaque cover, and a cork plunger for pushing the flies down into one end of the tube.   On the left, a box holding an electric light, and an isolation vial and box in which flies are kept previous to testing.

other, the handling of the tube and the raising of the barrier.   In comparisons, the mean and variance of ten consecutive trials in one group were matched against similar results taken immediately afterward from the other group.

DATA

TABLE 1

NORMAL ALLELOMORPH OF *bw* BACKCROSSED INTO BROWN STOCK.   FIRST SERIES, 12–13 CROSS-OVER GENERATIONS.   TIME IN SECONDS

	Brown *bw/bw*				Red *bw/+*		
n	Mean	Variance	SD	n	Mean	Variance	SD
10	15.27	2.54		10	17.32	4.17	
10	11.29	.93		10	11.14	2.41	
10	9.58	.39		10	12.53	3.05	
10	12.28	.77		10	10.14	1.74	
40	12.11	5.31	2.30	40	12.78	10.49	3.24
	$M_{red} - M_{brown} = .67 \pm .64$				$SD_{red} - SD_{brown} = .94 \pm .44$		

The first comparison between homozygous (brown) and heterozygous (red) flies in the brown stock (Table 1) showed a small but significant difference in variability as measured by the standard deviation.   There was also a difference in the mean, but not large enough to be significant.   The backcrossed flies were lost before a long series of experiments could be done, and the normal allelomorph was again crossed into the brown stock.

TABLE 2

NORMAL ALLELOMORPH OF *bw* BACKCROSSED INTO BROWN STOCK. SECOND SERIES, 13–17 CROSS-OVER GENERATIONS

Brown *bw/bw*				Red *bw/+*			
n	Mean	Variance	SD	n	Mean	Variance	SD
9	10.90	.63		10	11.53	.27	
10	9.50	.64		10	9.90	1.03	
10	12.59	1.17		9	11.51	.97	
10	10.42	.24		9	10.97	2.61	
10	10.05	1.03		10	10.96	1.14	
10	12.72	.99		10	13.36	2.12	
9	13.77	2.31		8	12.28	1.62	
68	11.40	3.04	1.74	66	11.48	2.58	1.61

$M_{red} - M_{brown} = .08 \pm .29$ $SD_{brown} - SD_{red} = .13 \pm .21$

In this second experiment there was no significant difference between the two types of flies. It can be concluded that in this stock the brown eye color probably had no important effect on the reaction to light and vibration.

TABLE 3

NORMAL ALLELOMORPH OF *w* BACKCROSSED INTO WHITE STOCK. 19–24 CROSS-OVER GENERATIONS

White *w/*				Red *+/*			
n	Mean	Variance	SD	n	Mean	Variance	SD
9	19.30	9.70		9	30.30	175.97	
10	21.08	17.34		10	34.04	483.08	
9	22.37	37.81		9	29.74	245.27	
9	22.86	87.44		9	39.88	533.81	
10	20.46	10.39		9	25.97	72.77	
47	21.19	35.55	5.79	46	32.03	327.89	18.10

$M_{red} - M_{white} = 10.84 \pm 2.84$ $SD_{red} - SD_{white} = 12.31 \pm 1.98$

In the meantime a similar experiment had been done within the white stock. After a large number of opportunities for crossing-over, important differences in the mean and standard deviation for white and red-eyed flies were found, and these effects may be attributed to the section of the chromosome which remained attached to the *w* gene, and probably to that gene itself.

In short, the brown and white-eyed stocks showed very great differences in behavior under comparable conditions. The brown-eyed flies were about ten seconds faster than the white on the average, and showed very much less variability. As a working hypothesis it might be supposed that the differences were caused by the brown and white eye colors or some other effects of the *bw* (brown) and *w* (white) genes. If this were true it would be expected that removing these genes would make the two stocks alike. No such result was obtained. Red-eyed flies of the brown

stock were almost exactly the same as the browns, and red-eyed flies of the white stock were even slower than the whites.

DISCUSSION

These results seem to indicate that an important effect on behavior can be produced by a single gene. However, as Schwab (1940) and others have pointed out, it is almost impossible to be sure that modifying factors on the same chromosome have not been carried along with the white gene. Even with twenty generations of backcrossing there is a good chance that no loss of genes has taken place within a chromosome distance corresponding to a five per cent. cross-over value.

On the other hand, there are good reasons for supposing that the white gene is itself responsible for the effect. Located within one and a half units of the end of the chromosome, a large number of linked genes could be carried only on one side of it. Furthermore, there is a ready explanation for the effect on behavior. According to McEwen (1917), the pigment in the eye of Drosophila is so placed as to cut out some of the light which enters each facet. A colorless eye should transmit more light than a red one, and since the speed of walking toward a light is proportional to intensity (Cole, 1922), a white-eyed fly should be faster than a red-eyed one. Even without these considerations, it can be said that a relatively short piece of the first chromosome adjacent to white can produce an important effect on behavior.

In fact, this segment of the chromosome may make the difference between flies giving a reaction to light and remaining inactive, as in many cases the red-eyed flies did not crawl at all. The conditions under which the experiment was done (a minimum amount of vibration and a weak light) appear to have been very close to a threshold point for this stock, below which the flies would not react. If so, a small genetic lowering of the reaction time in the red-eyed flies would account for the great lowering of the average time and large increase in the amount of variability observed. It thus appears that the importance of the effect was partly dependent on the presence of a threshold. Such a condition would, of course, magnify any differences, genetic or environmental.

It is obvious that more than one genetic factor can modify the behavior of Drosophila. The original red-eyed flies which were crossed into the white stock were faster than the white (Mean

17.89 ± 0.59, SD 3.25 ± 0.42), but after repeated back-crossing were slower than the white. This sort of result has been described by Dobzhansky (1927) and Schwab (1940) in studies on spermatheca shape, and it may be explained by the presence of another factor or factors which also speeded up the reaction and which were eliminated by back-crossing. And there must have been still another factor or factors which would account for the difference between the brown and white stocks.

The data are limited by the difficulties and laboriousness of the method. Nevertheless, a general picture of the causes of variability in the photic reaction in Drosophila can be painted with some degree of certainty. There are probably several independent genetic factors, some tending to increase the speed of reaction and some to decrease it. There are also several variable environmental factors, such as light, vibration, temperature and conditions of nutrition. The importance of the effects of these various causes may be magnified near a threshold. If both were uncontrolled, an extraordinary multiplicity of effects would be expected. This is very different from the interpretation of insect behavior which was given by early naturalists such as Fabre.

Even when the same group of flies was tested and hereditary differences could not affect the data, and when every effort was made to keep environmental conditions the same, a great deal of variability was observed. This can be seen in the tables. If hereditary variability (which is probably more important in laboratory raised flies) is added to this, it can only be concluded that Drosophila is capable of a great deal of individual variation and adjustment in its behavior.

As to the original problem of the importance of hereditary variation, a large effect on behavior can be produced by a relatively small piece of a chromosome and probably by a single gene. This indicates a general possibility which can only be disregarded when uniform heredity is secured by inbreeding or other means.

It has been shown that the large effect of the white gene was probably dependent on a set of environmental conditions involving a threshold. On the other hand, the effects of minor environmental changes are dependent on heredity, since they produced a large amount of variability in series of tests on the same red-eyed flies and a much smaller amount in corresponding tests on the whites. With this interdependence of heredity and environ-

ment it is impossible to give any general rule as to their relative effects. It is enough to say that heredity *can* be important, and that genetic factors are apt to increase in relative importance as environmental conditions are made constant.

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HE RATE OF ROOT ELONGATION IN DIPLOID AND TETRAPLOID SUDAN GRASS AND RYE

It is common knowledge that the artificially produced tetraploids in grasses, at least, are slower growing than the diploids from which they are derived. In fact, it is almost the rule. However, no information is available for the rate of elongation, *i.e.*, growth, of roots from germinating seeds for a comparison of diploid and tetraploid lines of the same species.

Seed of tetraploid Sudan grass was obtained from Dr. R. J. Garber, of the U. S. Regional Pasture Research Laboratory, State College, Pennsylvania, and seed of tetraploid rye was obtained from Dr. E. Dorsey, Cornell University. The Division of Entomology of the University of Minnesota cooperated by making available controlled temperature chambers. Only the diploid rye is of the same variety as the tetraploid.

All seeds were soaked for an hour and germinated on moist filter paper at a number of different temperatures. Measurements were taken in millimeters every 12 hours for three days. The results of this experiment are found in Tables 1 and 2.

At all temperatures, excepting 40° C., the roots of tetraploid Sudan grass elongated at a faster rate than the diploid Sudan grass. The diploid rye roots grew faster than the tetraploid rye at all temperatures. These differences were also reflected in the

TABLE 1

Sudan Grass (*Sorghum sudanense*). Total Periodic Growth and Periodic Increment in Millimeters Per 12-Hour Period

		1	2	3	4	5	6
Total Periodic Growth							
20° C.	2n	–	.2	.3	1.5	4.3	6.8
	4n	–	.8	1.5	4.6	9.6	14.7
25° C.	2n	.2	1.5	6.8	14.8	24.0	34.0
	4n	.6	3.1	11.9	24.5	36.7	50.0
30° C.	2n	.5	8.3	22.0	32.4	38.8	45.3
	4n	.9	14.9	38.7	53.3	63.5	74.1
35° C.	2n	1.1	12.2	25.2	34.0	40.5	45.9
	4n	2.2	21.3	46.9	60.8	71.0	83.4
40° C.	2n	.6	2.9	9.0	14.6	22.7	25.8
	4n	.6	4.0	9.4	15.8	20.8	24.8
Periodic Increment							
20° C.	2n	–	.2	.2	1.1	2.8	2.5
	4n	–	.8	.8	3.0	5.0	5.1
25° C.	2n	.2	1.3	5.3	7.6	9.2	10.0
	4n	.6	2.5	8.8	11.1	12.2	13.3
30° C.	2n	.5	7.8	13.8	10.4	6.4	6.5
	4n	.9	14.0	23.8	14.6	10.2	10.6
35° C.	2n	1.1	11.1	13.0	8.7	6.5	5.4
	4n	2.2	19.1	25.6	13.8	10.2	12.4
40° C.	2n	.6	2.3	5.9	5.7	5.2	3.1
	4n	.6	3.2	5.3	6.4	4.2	4.1

TABLE 2

Rye (*Secale cereale*). Total Periodic Growth and Periodic Increment in Millimeters Per 12-Hour Period

		1	2	3	4	5	6
Total Periodic Growth							
15° C.	2n	–	–	–	2.4	5.3	7.9
	4n	–	–	–	1.2	4.3	7.1
20° C.	2n	–	.9	5.0	9.8	14.1	19.7
	4n	–	.5	3.7	8.4	12.2	17.3
25° C.	2n	–	2.8	8.8	15.8	24.1	34.9
	4n	–	2.1	7.6	14.1	21.7	33.8
30° C.	2n	.7	4.7	11.7	19.5	25.3	31.5
	4n	.4	2.2	6.9	13.7	20.9	31.4
35° C.	2n	–	4.3	10.4	15.4	18.1	22.7
	4n	–	.9	4.0	7.2	11.7	17.0
Periodic Increment							
15° C.	2n	–	–	–	2.2	3.1	2.6
	4n	–	–	–	1.2	3.1	2.9
20° C.	2n	–	.9	4.1	4.8	4.3	5.8
	4n	–	.5	3.2	4.6	3.9	5.1
25° C.	2n	–	2.8	6.0	6.9	8.3	10.8
	4n	–	2.1	5.6	6.5	7.6	12.1
30° C.	2n	.7	4.0	7.0	7.8	5.8	6.2
	4n	.4	1.8	4.8	6.8	7.2	10.5
35° C.	2n	–	4.2	6.3	5.2	2.7	4.6
	4n	–	.9	2.9	3.2	4.6	5.3

rate of germination of roots.   The rye data are characteristic of those found for other grasses where the tetraploids are slower growing than the diploids.   The Sudan grass data are the first known to the writer where the tetraploid is faster growing than the diploid, at least for grasses.   A possible explanation may be found either in a difference of auxin production or of auxin utilization for the tetraploid compared with the diploid.   The change from diploidy to tetraploidy may have a different effect on auxin relations in the Sudan grass than in the rye.

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IMMUNOLOGY AS A TOOL IN BIOLOGICAL RESEARCH[1]

IMMUNOCHEMICAL APPROACHES TO BIOLOGICAL PROBLEMS

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I venture to address this gathering of geneticists and zoologists with an exhilaration engendered by a sense of the daring involved in an excursion into well-explored fields of knowledge remote from those into which my own work has extended. I trust, however, you will forgive this excursion or incursion, as it is intended more to remind you of progress already made in your fields along immunochemical lines, rather than to suggest the adoption of wholly foreign techniques and ideas.

However, before reviewing these applications, it might be well to describe again in modern chemical terms some of the concepts fundamental to immunology.

Knowledge of antigens, or the substances stimulating immune responses in animals, has been greatly extended in recent years. Thanks to chemical fractionations, the ultracentrifuge, the Tiselius electrophoresis apparatus and other powerful tools, one may no longer consider horse serum, for example, or an animal or bacterial cell, as "an antigen," but must recognize it as a collection of antigens,

each with distinct properties and potencies. Many immunological observations were and are difficult to interpret because this complexity was not taken into account. It is also apparent that many antigens are proteins and that most proteins are antigenic. Much work has been done showing that denaturation as well as introduction of the most varied chemical groupings at almost any point of substitution results in a definite change in immunological specificity.

Now most of you will remember that some time ago, in Avery's laboratory, we found that type specificity among the encapsulated bacteria depended upon another kind of antigen. This type specificity was due to a peculiar group of polysaccharides resistant to the usual sugar-splitting enzymes. The specific polysaccharide of each pneumococcus type, for example, was different from those of other types, and could be characterized by its distinctive physical and chemical properties. The sugars from types II and III pneumococcus were obtained free from nitrogen, and were the first instances in which immune specificity had been rigorously demonstrated in a class of substances other than proteins.

With this brief discussion of specificity as a basis, what can be said about the requisite conditions for antigenicity? It is obvious that we must have a complex structure and large molecules, and one of the important things seems to be the repetition of structural units. This is a highly probable consequence of the modern views of protein structure. We also know that the specific carbohydrate of type III pneumococcus, for instance, is made up of many cellobiuronic acid units. Some multiple of this unit must function as the immunologically reactive grouping, for when the carbohydrate is partially broken down by mild hydrolysis the fragments of two or more units still react in anti-pneumococcus type III horse serum. Therefore we may assume that in order to function fully as an antigen a substance of large molecular size must be of such nature as to allow repetition of cer-

tain structural units. Possibly for this reason ordinary lipids do not appear to have a clear-cut antigenic function.

I think Dr. Landsteiner would add that any simple chemical substance may also function as an antigen especially if the chemical properties are such as to allow its combination with protein to form new antigens. Complex structure is not necessary, therefore, if a number of molecules of a smaller entity can combine to form part of a larger structure.

With regard to antibodies, the immune substances engendered in animals as a result of the antigenic stimulus, we are in a position to be equally definite. Use of new quantitative chemical microanalytical methods made it possible to measure antibodies in sera in actual weight units. One could, for the first time, express antibodies in terms of specific nitrogen per cubic centimeter of serum, because after precipitation with a slight excess of antigen non-specific material could be washed out. Since the amount of nitrogen in the added antigen is known this may be subtracted and the residual nitrogen in the washed precipitate is due to the antibodies. Highly purified antibody solutions obtained as a consequence of information gained by these new methods were examined in the ultracentrifuge and electrophoresis apparatus and were shown to have the properties of typical serum proteins.

Buchner's hypothesis that antibody contained fragments of antigen was proposed at a time when the actual nature of antibodies was not understood. This hypothesis never appealed to the chemist because in a number of instances like repels like, rather than attracts. In 1932 Breinl and Haurowitz proposed a theory that antibodies are formed by a modification of the normal process of serum globulin synthesis as a result of penetration of antigen or specific portions of the antigen to the site of globulin synthesis. The disturbance so brought about influences the course of that synthesis in a sense characteristic of the antigen so that when the modified globulin appears in the circulation and again encounters the antigen

interaction is possible. This not very clear picture was later expressed in somewhat more definite form by Mudd. An extension of this hypothesis has recently been made by Pauling which is even more graphic and reasonable but as devoid of experimental basis as the Breinl and Haurowitz theory. The Pauling hypothesis carried a second idea—that if one could take normal globulin, denature it and fold it up again in the presence of antigen, artificial production of antibodies might be accomplished. Pauling now believes he has been successful in this, but such details of his experiments as have been published do not include complete controls. Burnet has recently proposed the origin of antibodies through modification by antigen of intracellular proteases which provide the framework for synthesis of partial replicas of themselves (globulins or antibodies). This would provide for antibody formation after destruction of antigen and for progressive changes in antibodies with successive immunizations.

These theories of antibody formation have been given a physiological basis in recent years by Dr. Florence Sabin as a result of experimental work with a red protein dye. Dr. Sabin has observed macrophages in the omentum and cells of the reticulo-endothelial system and found that, at a certain stage of development, surface layers which form folds waving back and forth finally disappeared as if they were being extruded from these cells. She believes this to be the source of serum globulins and that the presence of an antigen (for example, the red protein dye) results in the specific modification of these globulins into the appropriate antibody.

Now for a few applications to genetics and biology:

Nuttall's pioneer work on the mapping of biological relationships through the study of the interaction of animal sera with antibodies formed when these sera are injected into a standard animal such as the rabbit was most fruitful and has been extended by numerous workers. The immunochemist has shown that interpretation of the com-

plex findings is often simplified if a single protein is used, rather than serum, which we know to be a complex mixture of albumin, at least three globulins, complement with its four components, and other minor substances, all or most of which may function as antigens and cause overlapping or zone effects in reactions with antisera. Nor is it certain that precipitation in different antisera is always due to the same antigen when such a mixture is used.

An extreme instance of the simplification wrought by the use of pure, crystalline proteins was the demonstration by Landsteiner and myself of the non-identity or identity of the oxyhemoglobins of various species by a physical-chemical (solubility) method as well as by the serological technique. By use of the quantitative precipitin method, in which the amount of antibody nitrogen precipitated by a single purified antigen is measured, information as to species relationships may be gained that is unobtainable by qualitative measurements. In this way Stokinger and I were able to show the close relationship, but lack of identity, of sheep and bovine thyroglobulins, and to demonstrate that even this organ-specific globulin hormone possessed a species-specificity entirely independent of that of the corresponding serum globulins. With the same quantitative method Treffers, Moore and I were able to give a plausible explanation for the differences shown by normal horse γ-globulin and antipneumococcus horse γ-globulin in rabbit antisera to the antibody (horse).

It is not always necessary, however, nor is it necessarily an advantage, to study the immunological behavior of single antigens, as Irwin and his collaborators have shown in their intricate but clearly defined studies of the numerous gene-linked antigens of avian and mammalian red cells. Another fruitful immunological approach to genetic problems has been made by Tyler in his studies of the agglutination of sperm by egg substances of *Arbacia*.

The discovery of the specific polysaccharides of pneumococcus in Avery's laboratory and the recognition that these sugar derivatives are the determinants of type-

specificity in this and other groups of pathogenic micro-organisms have led to far-reaching results, most of which are beyond the scope of this lecture. However, Griffith's initially almost unbelievable discovery that one pneumo-coccus type could be converted into another has impor-tant implications, not only in carbohydrate chemistry and bacteriology, but in general biology and genetics as well. As many of you know, pneumococci of Type I, for example, may be degraded to a form devoid of type-specificity and then converted, theoretically, at least, back to the original type, or into any one of some forty-odd other types. This was originally accomplished by grow-ing the degraded cells in the presence of a heat-killed suspension of pneumococci of the type into which the liv-ing cells were to be converted. Studies by Avery, Daw-son and Alloway showed, however, that certain extracts of type-specific pneumococci contained a substance or sub-stances responsible for this conversion, and that the type-specific carbohydrates themselves were not the determin-ing factors. Thus, any pneumococcus cell is potentially able either to synthesize, one at a time, nearly fifty differ-ent specific polysaccharides or may be so influenced by a series of substances that such varied syntheses become possible. The immunochemist must leave it to the geneti-cist to decide whether or not these processes are true mutations, but I am happy to say that Avery is continuing the study of the transforming principle, and the eventual elucidation of its nature is certain to throw light on this and many other questions.

These are merely a few of the instances in which im-munological and immunochemical methods have provided an insight into biological mechanisms. To give a more complete summary would carry me far beyond the allotted time, but I hope you will recall other examples which I would have liked to mention. More important, however, I hope that those of you who may have required this re-minder of the possibilities of these powerful tools will consider them as aids in the solution of present and future biological problems.

EVOLUTION OF THE HUMAN BLOOD GROUP FACTORS[1]

DR. ALEXANDER S. WIENER

THE discovery of human blood groups by Landsteiner in 1900–01 served to explain unexpected fatal reactions resulting from transfusions of blood from one human being to another, only to pose the question, still unanswered, as to the nature of the blood group differences, their origin and function if any. This problem has become more acute during the past two decades with the discovery of additional agglutinogens in human blood, M, N, P and Rh, so that it is now possible without much difficulty to classify individuals into 72 classes, depending on what combination of factors is present in the blood.[2] Because of the limited space the discussion will be limited to the original four blood groups, the three M–N types, and the more recently discovered Rh factor.

The classic blood groups depend on the presence or absence of two agglutinogens A and B in the cells and two corresponding isoagglutinins α and β in the serum. The groups O, A, B and AB are determined by the four possible combinations of the two agglutinogens, A and B. Another characteristic property is the reciprocal relation between agglutinogens and agglutinins, those isoagglutinins invariably being present for the agglutinogens absent from the cells (Landsteiner's rule). Other striking features are the hereditary transmission of the groups by means of three allelic genes, *A*, *B* and *O*, and the differences in the distribution of the groups in different races. The most outstanding examples of the latter are the higher frequency of gene *A* as compared to gene *B* in white races in contrast to Asiatic peoples where gene *B* predominates, while in certain more primitive peoples

such as Australian aborigines, American Indians, Polynesians, etc., one or two of the three genes may be practically lacking.

A number of theories have been proposed to explain the present distributions of the blood groups in white races as well as the differences among various peoples. Because of the predominance of gene O in almost all races, it has been suggested that group O was the original group and genes A and B appeared in man later on and increased in frequency by mutation (Gates, 1936; cf. Snyder, 1929). Other investigators (e.g., Bernstein, 1932) believe that there were originally a number of isolated races, some exclusively or predominantly group O, others predominantly group A or group B, and that the present blood group distributions resulted from crosses between these races. Candela (1942), in fact, states that the present differences in blood group distribution, in particular the striking distributions in some primitive peoples, are merely remnants of this original condition. On the other hand, Boyd (1940) has suggested that primitive man started with a certain blood group distribution and that, as he spread to the "four corners of the earth," certain isolated groups lost entirely or largely one or two of the blood group genes, giving rise to the present differences in distributions of the groups. Which, if any, of these theories is correct is uncertain, but it might be remarked that some clues have been obtained from the extensive studies on the numerous peoples in Europe and Asia. These show a progressive decrease in the frequency of gene B as one proceeds from East to West (Hirszfeld and Hirszfeld, 1919), suggesting that group B was introduced into Europe from Asia. According to Candela (1942), this occurred during the Mongolian invasions between the fifth and fifteenth centuries, the gene being derived from the brachycephalic Central Asiatic Mongols, best represented at the present time by the Buriats and Kalmucks (cf. Bernstein, 1932). According to computations made by Wyman and Boyd (1935) and

Haldane (1940), if the blood groups attained their present frequencies mainly by mutation, then it would be necessary to postulate an improbably high rate of mutation, and it is more reasonable to ascribe the present distribution of the blood group genes to the effects of migration, isolation and inbreeding, and racial crossing.

I propose now to discuss the light thrown on these problems by studies in lower primates. For the discussion it is necessary to mention that in man the group factors A and B are not restricted to the blood cells but are also found in tissues, organs and secretions, except that in certain individuals (known as non-secretors) the group substances are absent from the secretions.[3] Studies on saliva, the most convenient material to test, show that this character is determined by a pair of allelic genes, S and s, located in a different pair of chromosomes from the blood group genes (Schiff and Sasaki, 1932). Also, in groups A and AB subgroups have been identified, based on the existence of two main varieties of A agglutinogen, A_1 and A_2, determined by two corresponding allelic genes. Studies on the distribution of subgroups and secretor character to date have been relatively few, but the results already obtained indicate their value as additional means of tracing racial relationships.

In the first systematic study on anthropoid apes, Landsteiner and Miller (1925a) succeeded in demonstrating that the blood of chimpanzees, orang-utans and gibbons could be divided into four groups indistinguishable serologically from the human blood groups, while such blood groups apparently did not exist in monkeys or lower animals. These results are in accord with the close kinship between man and apes and at the same time furnished a striking instance of biochemical evolution. Moreover, they seemed to cast further doubt on the mutation theory already discussed, unless one was willing to assume the occurrence of parallel mutations in man and ape.

[3] In group O individuals, the two types are distinguished by testing the saliva with so-called anti-O sera.

Subsequent studies by Landsteiner (1928a), Troisier (1928) and others served to confirm and amplify these findings and at the same time revealed striking differences among the apes in the distribution of the groups. Of 92 chimpanzees examined, 81 belonged to group A, only 11 to group O, none to group B or group AB. The orangs and gibbons, on the other hand, all belonged to groups A, B or AB, so that gene *O* was apparently lacking from these species. The lack of adequate information concerning gorillas is understandable, but recently material from a dead gorilla was made available to Candela and myself. The blood serum of this animal contained anti-A but not anti-B agglutinins, but the blood cells failed to react distinctly with either anti-A or anti-B sera, in apparent conflict with Landsteiner's rule as it holds in human beings. It then occurred to us to test the salivary glands, and in this way the presence of the B substance was readily demonstrated, accounting for the absence of anti-B agglutinins from the serum (Candela, Wiener and Goss, 1940). Subsequently, additional gorillas were grouped by testing their saliva and urine; interestingly, all 13 lowland gorillas (*G. gorilla*) tested belonged or were related to group B, while 2 mountain gorillas (*G. berengei*) both belonged to group A (Candela, 1940).

While the older investigations on lower monkeys did not reveal the presence of groups corresponding to the human blood groups,[4] tests on their blood sera demonstrated the existence of certain regularities. For example, the sera of rhesus monkeys always contain anti-A but not anti-B agglutinins, while sera from vervet monkeys contain anti-B but not anti-A (Landsteiner, 1928b). Tests on the blood cells failed to account for this phenomenon, but in view of the results obtained in gorillas, it occurred to the writer that the explanation for the phenomenon might be found by testing the organs and secretions. Wiener, Candela and Goss (1942) then found that

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[4] It should be mentioned, however, that Landsteiner and Miller (1925b) did demonstrate the presence of B-like antigens in the erythrocytes of New World monkeys but not in Old World monkeys.

the secretions and organs of rhesus monkeys regularly contained the group substance B. By extending the tests to other monkey species, it was established that in general, in monkeys and gorillas as in other apes and man, Landsteiner's blood group rule holds, but the reciprocal relationship exists between agglutinins in the blood serum and antigens in the organs and secretions, rather than the blood cells. Accordingly, group substances are present in organs and secretions in lower primates, while their presence in the erythrocytes appears to be a more recent development in evolution.

Gene O appears to be rare in monkeys as well as apes, because in the admittedly small series of tests carried out to date only a single monkey giving reactions corresponding to group O was encountered, and in this instance one can not entirely exclude the possibility that the monkey was a non-secretor. This is in striking contrast to the situation in man where gene O has the highest frequency, and may suggest that instead of O being the original group, genes A and B came first and that gene O arose later by mutation. If this assumption is correct, then it is more likely that gene O arose from gene A than from gene B. This follows from the existence in some races of man and in chimpanzees of groups A and O alone, to the exclusion of gene B. Moreover, the varieties of A agglutinogen, A_1, A_2, A_3, ... form a graded series which in serological tests react progressively less intensely with anti-A sera and more intensely with anti-O sera.[5]

The distribution of the blood groups in monkeys is similar to that of apes in that in monkeys of a single species not all four groups are represented. In man, also, in Paleolithic times when man was presumably a comparatively rare animal, isolation and inbreeding of small numbers of individuals may have occurred, giving rise by chance to populations lacking one or more of the blood groups. The present distributions of the blood group genes, in the writer's opinion, can be explained in part

[5] This graded series of genes has also been discussed by Hirszfeld (1938), who, however, believes that gene A arose from gene O.

by crossing of two or more such populations with differ-
ent blood group distributions (as Bernstein and Candela
believe), and in part to subsequent migration to distant
parts of the world of small groups (as Boyd believes)
with chance loss of one or more of the blood group genes.
As Wyman and Boyd remarked, this would explain the
similarity in blood group distribution of peoples geo-
graphically distant from one another.

It may be well to point out at this time that the fact that
blood or secretions from apes, monkeys and man give
identical or similar reactions with anti-A and anti-B test-
ing sera does not necessarily establish the presence of
identical substances in these species, but merely of chem-
ically related substances.   Though it is difficult to test
the point, presumably the substances in apes which give
group-specific reactions are more closely related to the
human group substances than those present in monkeys.
In fact, substances serologically related to the group sub-
stances A and B have even been found in the blood, organs
and secretions of certain lower mammals.   To be sure, in
these lower species the reciprocal relationship between
group substances and agglutinins in the sera appears to
be less striking, but it is not entirely certain how signifi-
cant this is, because the tests to date have mostly been
carried out with reagents prepared from human blood.
Incidentally, the blood group rule has practical applica-
tion, for example, when selecting rabbits for the produc-
tion of immune anti-A sera, in that rabbits lacking sub-
stances giving A reactions produce far more potent anti-
sera than those possessing such substances.

Studies on the properties M and N (Landsteiner and
Levine, 1928a, b) of human erythrocytes have furnished
further data concerning biochemical evolution.   In man
these properties are transmitted by a pair of allelic genes,
M and N, located in a different pair of chromosomes from
A, B, O, and they give rise to three types of blood, M, N
and MN.   Unlike A and B, however, factors M and N ap-
pear to be confined to the erythrocytes.   In most white

races the distribution of the types is approximately 30 per cent. M, 20 per cent. N and 50 per cent. MN, so that gene *M* is slightly more frequent than gene *N*. American Indians and Eskimos are characterized by a high frequency of gene *M*, while Australian aborigines and Ainu have a high frequency of gene *N*. Besides confirming the relationship between American Indians and Eskimos, on the one hand, and Ainu and Australian aborigines, on the other, these results suggest that the present distribution of the M-N types among white individuals may have arisen from crossing some time in the past of two or more races, some with high frequencies of gene *M*, others with much gene *N*. These results, accordingly, are in line with our conclusions from studies on the factors A and B.

Factors corresponding to M and N have not been found in the blood of lower animals. Their presence in the blood of anthropoid apes was first reported by Landsteiner and Levine (1928b), but for some time thereafter contradictory results were obtained with monkey blood. In testing for property M and N, it occurred to the writer to examine monkey and ape blood with a variety of antisera. It was then found that the irregular results previously reported were due to qualitative differences in the M antisera, even though they all gave parallel reactions on human blood; for example, some anti-M sera agglutinated the blood of rhesus monkeys while other anti-M sera gave no reactions. Tests were then carried out by Landsteiner and Wiener (1937) and Wiener (1938) with a variety of anti-M sera on a number of species of apes and monkeys, with the results given in Table 1. It was found that several different M-like antigens exist in the blood of monkeys and apes, so that the species could be arranged in a graded series based on the resemblance of their blood to human M blood. As expected, the blood of anthropoid apes resembled human M more closely than monkey blood, chimpanzees' blood being the most like the human. Among the monkey species, M agglutinogens were regularly found in the Cercopithecidae but were found in only

one of the species of Platyrrhinae (and in this case the blood reacted only with a special serum), suggesting that the former are higher in the evolutionary scale.

In tests made with anti-N sera, positive reactions have thus far been obtained only with blood from chimpanzees (Landsteiner and Levine, 1928b; Wiener, 1938). Not all anti-N sera react with chimpanzee blood, indicating that the N factor in the ape blood (like the M factor) is related

TABLE 1

M AGGLUTINOGENS IN MONKEY BLOOD

Source of Blood Specimens	Anti-M Testing Fluid*					
	M5	M1	M21	M35	M2	M82
Human M	+ + +	+ + +	+ + ±	+ + ±	+ + ±	+ + ±
Human N	0	0	0	0	0	0
Chimpanzee	+ + +	+ + +	+ + +	+ +	+ + +	±
Old World Monkeys (Cercopithecidae)						
Sphinx Baboon	+ + +	+ +	+ + ±	0		0
Drill Baboon	+ + +	+ + +	+ + ±	(±)	(+)	(+ ±)
Chacna Baboon	+ + +	+ + +	+ + ±	0	tr.	0
M. rhesus	+ + +	+ + +	+ + ±	(+ ±)	±	0
Java Macaque	+ + +	+ + +	+ ±	0	0	0
Sooty Mangabey	+ + +	+ + +	+ + ±	tr.	±	±
Green Monkey	+ + +	+ + +	0	0	0	0
New World monkeys (Platyrrhina)						
White Spider Monkey	+ + ±	0	0	0	0	0
Black Spider Monkey	±	0	0	tr.	0	0
Wooly Monkey	0	0	(±)	0	0	0
Brown Ringtall (Capuchin Monkey)	0	0	0		0	0
Moss Monkey	0	0	f.tr.	±	0	0
Lemur	0	0	0	0	0	0
Average Titer of Testing Fluids	64	64	32	24	16	16

Of several species two individuals were tested, of brown ringtails 4, of *Macacus rhesus* 45.

Reactions placed in parentheses were found not to be removed by absorbing the sera with human M blood. In addition there are a number of weak reactions which were not tested with human M blood but probably belong to the same category.

* These were prepared from the immune sera in the usual way, by diluting with saline and then absorbing with packed human N cells.

to but not identical with the corresponding property in human blood. Interestingly, all chimpanzees thus far tested have been found to possess both M and N, while if these were contrasting characters as in man, only half of the apes should have the two factors together. Perhaps, in these apes the reactions for M and N may both be due to a single chemical substance and the discrete properties M and N of man may possibly have arisen by

mutations in opposite directions from some such common antigen.

Based on the demonstration of M-like agglutinogens in monkey blood, Landsteiner and Wiener (1937) attempted the production of anti-M sera by immunizing rabbits with rhesus blood, and found that potent anti-M immune sera could be obtained in this manner. Similar results were obtained by Wheeler and Stuart (1939). It was considered that in the same way it might be possible to obtain type specific immune sera for human blood against factors hitherto unknown, if such factors were present in monkey blood. In fact, Landsteiner and Wiener (1940, 1941) then succeeded in obtaining anti-rhesus immune sera (first in rabbits, then much more readily in guinea pigs), which reacted with about 85 per cent. of bloods from white individuals. The factor present in human blood of the former type was designated as Rh, to indicate the manner in which it was first detected. The agglutinogen Rh was subsequently found to be of clinical importance, on account of its role in the pathogenesis of intragroup hemolytic reactions (Wiener and Peters, 1940; Wiener, 1941) and in a disease of the newborn known as erythroblastosis fetalis (Levine *et al.*, 1941).

As already mentioned, it has not yet been possible to ascribe any physiological function to the blood group factors. The Rh factor is the first thus far discovered which has proved to have a definite, though weak, selective effect, because it can be responsible for stillbirths or neonatal deaths, as pointed out above. In the matings in question, the mother is Rh-negative, the father Rh-positive, and the fetus in utero Rh-positive, having inherited the factor from the father. Due presumably to some defect in the placenta, some of the fetal blood passes into the maternal circulation, and in susceptible women this results in the production of anti-Rh isoantibodies. The Rh isoantibodies then filter through the placenta and destroy the blood of the fetus either in utero or shortly after birth, giving rise to symptoms of the disease. It may be of

interest to mention that in one of ten to twelve matings the mother is Rh-negative and the father and child Rh-positive, while the incidence of the disease is only about 1 in 400, indicating that only about one in forty women is capable of being sensitized.

It can be shown (Wiener, 1942) that in populations where the genes *Rh* and *rh* are equal in frequency—Landsteiner and Wiener (1941) have shown that the Rh factor is transmitted as a simple mendelian dominant by a pair of allelic genes, *Rh* and *rh*—the death of infants and fetuses with erythroblastosis will have no effect on the relative distribution of the genes. When the incidence of the genes is unequal, however, there will be a definite selective action against the less frequent one. It is of interest to note that certain populations have been found, *e.g.*, American Indians (Landsteiner, Wiener and Matson, 1942) and Chinese (Levine, 1942), in which the Rh-negative type is practically lacking. It is difficult, however, to explain the high incidence of the recessive gene (almost 40 per cent.) in white populations. Gates's theory proposed for the blood groups if applied to the Rh factor, that the present distribution was built up by repeated mutations from *Rh* to *rh*, can immediately be excluded because the selective action of isoimmunization would certainly nullify the effect of such mutations. Again we must fall back on the theory of two or more original races, predominately Rh positive or negative, respectively, the present distribution being explained by crossing of these races some time in the past.

Some remarks on the relationship between species-specific and type-specific antigens are pertinent. It is significant that certain factors characteristic of the individual in man are shared by all members of other species. For example, the so-called F_A antigen is present only in human blood of groups A and AB but appears to be shared by all sheep, and M antigens have been found in all rhesus monkey blood thus far examined. In doves and pigeons, Irwin *et al.* (1936, 1937, 1940) have demonstrated that the

species-specificity of the blood is determined by a number of agglutinogens shared perhaps by all members of the same species, and in species crosses the genes for these agglutinogens undergo independent assortment giving rise to a large number of individual blood differences in subsequent hybrid generations. In this connection, as already mentioned, members of the species of monkeys thus far examined have been found to belong predominantly to a single blood group, suggesting again that the four blood groups possibly arose by crossing (*cf.* Landsteiner and Miller, 1929a).

In conclusion, the observations on the blood groups factors in apes and monkeys indicate that biochemical evolution has roughly paralleled morphological evolution. While no function has yet been established for the individual blood antigens, the observations on isoimmunization in pregnancy are of interest. No evidence is available as to whether in certain cases such a mechanism might perhaps interfere with the viability of the offspring of species crosses. Future investigations will undoubtedly throw further light on this intriguing problem.

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IMMUNOGENETIC STUDIES OF SPECIES RELATIONSHIPS[1]

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NEARLY all biologists are aware that the branch of science called "immunology" had its origin in the study of reactions to infectious diseases. The concept that each disease was the result of the interaction of the host and a particular infecting agent was followed shortly thereafter by the knowledge that there was a specificity in the resistance produced by an infection. Studies to determine the nature of this striking phenomenon gave birth to the science of immunology. Whether this once lusty infant is now approaching vigorous maturity is not a subject of discussion at this time. However, we may definitely state that this one-time infant prodigy has long since weaned itself from its mother-science, bacteriology, and has embarked on an independent career which has given much, and promises more, in explanation of the specificities of biological reactions.

Another egg cell, which later developed into the scientific discipline now called "genetics," was fertilized at about the same time as that of bacteriology, the mother-science of immunology. The infant developing from this fertilization was given no nourishment at birth and promptly went into a state of coma which lasted for several decades. Three "wise men" of our own time discovered the infant in this state and, upon receiving proper attention and adequate nourishment, it made a phenomenal growth. There have been a few persons who predicted a "bad end" for the youngster, but *he* has managed to survive and *their* voices are stilled.

Actually, the growth periods of these two branches of science are relatively equal. Moreover, while immunol-

ogy sprang directly from bacteriology, genetics had two parents, botany and zoology. A genealogist would of course remind us that these parent branches are but parts of a single stem, biology, making the relationship of two of the disciplines which are being discussed to-day as that of first cousins. Certainly both of these cousins have contributed greatly to our conception of specificity in biological reactions, a goal towards which biological research in general is ever striving. If each of these by itself is able to make significant contributions to our understanding of biological phenomena, surely much could be anticipated from their combined efforts.

One of the first joint attacks of these two branches of science was made in the study of antigenic characters in the red blood cells of humans, the discovery of which was made by Landsteiner (1900, 1901). The same worker was also first to use the antigens of the blood cells in a comparison of the relationships of two species and their hybrids (Landsteiner and Van der Scheer, 1924), in contrast to earlier immunological studies of species relationships of animals in which serum had been employed almost exclusively. These findings, in conjunction with those obtained by Heidelberger and Avery (1923, 1924) which showed that the immunological specificities of the different types of pneumococci were amenable to a chemical explanation, suggested some years ago that the combined use of these two disciplines might well yield fruitful results with suitable experimental material. Although certain plant material appeared at the outset—and still does—to be favorable for the proposed experiments, the various species and backcross-hybrids which Professor L. J. Cole had previously produced at the University of Wisconsin seemed tailor-made for such a study. All these were generously placed at our disposal.

In making a study of genetic relationships between species it would undoubtedly be of advantage to use characters whose expression is not affected by different genetic complexes. The antigens of the red blood cells

appear to be an example of such characters. The rela-tively small number of cell divisions obtaining between the fertilized egg and the laying down of the mother cell of the tissue which forms the blood cells is one line of evidence which makes it reasonable to assume that the cellular antigens are the more or less direct products of their respective causative genes. Indeed, Haldane (1938) has postulated that "the gene is a catalyst making a particular antigen, or the antigen is simply the gene or part of it let loose from its connexion with the chromo-some." However, the findings that antigenic characters, either in species hybrids (Irwin, 1932) or within a species (Thomsen, 1936), may be the result of complementary action of genes, show that interaction is sometimes pos-sible between genes affecting these substances. Hence a sweeping statement can not be made that there is but one step from gene to antigen. Nevertheless, other than these few examples of complementary interaction, it appears that in general the genes with antigenic effects produce the same result irrespective of the other genes present. As Wright (1942) has stated, "Here we seem to have epigenesis at its simplest."

The technical procedures used in our laboratory in making comparisons of various species of birds have been described elsewhere in detail (Irwin and Cole, 1936; Irwin, 1939). Briefly, it can be said that a definite dif-ferentiation of the cells of any pair of related species of pigeons and doves has been possible only after the anti-serum to one has been absorbed by the cells of the other. For example, when antiserum to Pearlneck (*Streptopelia chinensis*) has been exhausted of part of its content of antibodies by mixing it with an excess of the cells of Ring dove (*St. risoria*), it becomes a "reagent" which will agglutinate the cells of Pearlneck, but not those of Ring dove. The antigens in Pearlneck reactive with this re-agent have been called the "species specific" characters of Pearlneck. This procedure has been portrayed dia-grammatically (Cumley and Irwin, 1941), and a schematic

representation of the antigens of Pearlneck, Ring dove and their F_1 hybrid as determined by these methods has been made (Irwin and Cole, 1936).

By virtue of the results from comparisons of the cells of many pairs of species of birds by such tests one may conclude·that, for any pair of related species, each possesses two kinds of cellular antigens, *viz.*, (a) those which are peculiar to one or the other of the species, and (b) those which are shared by both.

In so far as hybrids have been produced between various species, they invariably have been found to possess all or nearly all the cellular components particular to both parental species, and all those shared by the parents (except for differences between the hybrids attributable to heterozygosity of one or both parents for species-specific or common components, or both). Also, hybrids between certain species only, not all species-hybrids, possess an antigenic complex which was not found in either parental species. This new or "hybrid" substance presumably is produced by the interaction of genes which in each parent produce only species-specific antigens, if any at all.

On the assumption that the *species-specific* and *common* antigens are gene-determined, definite ratios of the antigens specific to one species would be expected in the offspring of backcrosses of the species hybrid ·to the other parental species. (Obviously, the genes which produce the antigens peculiar to either species are simplex in the species-hybrids.) For example, if there were but one cellular component which differentiates the first from the other species, two kinds of progeny from mating the species hybrid to the second species would be expected in equal numbers—those with and those without the character. If there were two such characters, and their causative genes were on separate chromosomes, approximate equality for four types of backcross progeny from the same kind of mating would be expected—those with both components, those with only one, those with the other

and those with neither. With any number of antigens, the number of types of offspring in the first backcross generation would be 2^n, in which n equals the number of species-specific antigens.

Antigens of Red Blood Cells

A. *Segregation of species-specific antigens in backcross progeny.* To use a specific example, offspring have been obtained by backcrossing, to Ring dove, species-hybrids from matings between Pearlneck and Ring dove. As yet only Pearlneck males have produced hybrids in this cross, and only hybrid males have produced backcross progeny. These circumstances rule out the possibility of cytoplasmic influence on the segregating Pearlneck antigens, since, as is generally admitted, any such influence would be transmitted through the cytoplasm of the egg.

Approximately ten cellular antigens peculiar to Pearlneck have been isolated in unit-form as a result of their segregation in progenies of various backcross generations (Irwin, 1939). These cellular characters have been called d–1, d–2, d–3 . . . d–12, the letter "d" indicating that the characters are found in doves. The genetic test of the unitary nature of each of these antigenic components has been that any backcross bird containing only one of these specific Pearlneck characters should produce but two kinds of progeny in matings to Ring dove; *viz.*, those with and those without the particular substance. That each of these characters, as carried by the backcross birds, is definitely different from the others may be shown by an extension of the immunological technic which permits the differentiation of the cells of one species from those of another. Such tests have been described previously (Irwin, 1939) and also presented diagrammatically (Cumley and Irwin, 1942a).

Although the proportions of backcross offspring with and without the respective characters peculiar to Pearlneck approximate those expected if each were the product

of a single gene, it is possible—and to us it seems highly probable—that most if not all of these specific antigens may be the result of the combined action of several linked genes on the particular chromosomes.   Usually in genetic parlance a heritable character which seemingly behaves as a unit is said to be produced by a single gene until evidence is obtained that more than one gene is involved. However, despite the resemblances the unit characters of each of these specific characters, in terms of genetic ratios, it is known that some of them are complex antigenically, and probably also genetically.   One explanation of the known complexity of at least two of these antigenic components (d–6 and d–11) of Pearlneck is that two or more genes are acting together to produce each (Irwin and Cole, 1940).   Unpublished data indicate that a parallel situation obtains for others of the characters.

What really has been accomplished by the genetic segregation of these antigens in the backcross generations parallels the fractionation of the antibodies in an immune serum by antibody-absorption.   Furthermore, the experimental results are in complete accord with the concept long held by immunologists that there are multiple antigens in cells, and that the various antibodies engendered by an animal during immunization with these cells are highly specific for the respective antigens.

Some unpublished evidence indicates rather strongly that the sum of these ten or so cellular characters now demonstrable equals the total complex of specific Pearlneck substances which are found in the species-hybrids. One can not, however, eliminate the possibility that antigens particular to Pearlneck other than these ten may exist, but, if so, it is probable that they are not detectable at the threshold of reaction (*i.e.,* at the dilution of the reagents) at which the tests have been made.   The demonstrable characters (specific to Pearlneck) may then be spoken of provisionally as "major" characters, in contrast to others detectable only at lower dilutions of the antiserum, which might be called "minor" characters.

These two species are readily differentiated by certain visible external characteristics. To date, critical tests have not been made of a possible correlation in backcross generations between the antigenic and the external characters that distinguish Pearlneck from Ring dove. Such a study does not appear promising at the moment, primarily because most of the visible characters of these two species intergrade to a considerable extent. An interesting observation has been made, however, by Shrigley (1940), that various types of abnormalities of the sperm, occurring infrequently in both these species, were markedly increased in the species hybrids. Furthermore, there was a definite tendency for backcross individuals which possessed a complex of antigens peculiar to Pearlneck to have a higher proportion of abnormal sperm than did the backcross birds lacking these properties. The backcross birds in the latter class were biochemically more like Ring dove than were the others, as were the proportions of abnormalities of their sperm. These results suggest a disharmony between at least certain of the genes of Pearlneck and those of Ring dove, which is reflected in the sperm. Other studies dealing with morphological and physiological differences between these species are indicated.

It is unfortunate that cytological studies of these backcross individuals, which might show a correlation of chromosome behavior with the cellular antigens, appear at the moment to be impractical, if not impossible. However, on the assumption that each of the ten characters in Pearlneck is produced by one or more genes on as many chromosomes, an estimate may be made of the relative proportion of the chromosomes of Pearlneck bearing genes for ''major'' cellular substances either specific to itself or common with Ring dove. The best cytological evidence suggests that there are approximately 30 pairs of chromosomes in pigeons and doves (Painter and Cole, unpublished data). Therefore, if one or more genes on at least many of these have a detectable effect on the

antigens of the blood cells, about one third of the total will carry genes with "major" effects distinguishing Pearlneck from Ring dove; all others with such effects will produce components *common* to the two species. Naturally, the proportion of the total chromatin material effecting either species-specific or common components need not necessarily parallel the proportion of the numbers of chromosomes with such diverse effects.

On the assumption that each of the genes producing cellular antigens particular to Pearlneck, as well as each of those producing the *common* substances, might have an allele with a different effect, the number of different combinations of antigenic characters within the species appears to be almost without limit. The greatest number of antigens known at present for any species has been found in this laboratory in cattle (Ferguson, 1941; Ferguson *et al.*, 1942), in which species more than 30 cellular substances have been demonstrated as probable units. The majority of these antigenic characters within the one species of cattle are detectable only at lower levels of reactivity (*i.e.*, at lower concentrations of antisera) than those which differentiate Pearlneck from Ring dove. They would then be classified as "minor" rather than as "major" characters, according to the grouping proposed earlier. (Unless the different cellular antigens which distinguish one species from another are of a different "order" than at least most of those which distinguish individuals within a species, the very anomalous situation presents itself in which individuals of the same species might differ in more antigens than do different species. That is, it has been found that the cells of Pearlneck differ from those of Ring dove by probably ten cellular antigens, whereas the cells of individual cattle—the same species—theoretically may differ by as many as 30 such antigens.) The genetic relationships of each of these 30-odd characters of cattle cells to the others have not yet been worked out in detail, but it seems probable that a marker has been provided for at least one member of

most of the 30 pairs of chromosomes of cattle as determined by Krallinger (1931). Furthermore, since no two of these antigens seem to have a simple allelomorphic relationship, the number of their possible combinations is well over a billion (2^n in which n equals 30, the number of different cellular characters). Thus, for this species and undoubtedly for many others, the biochemical specificity of the individual shows definite promise of being a reality, and not simply a possibility as yet unproven.

Furthermore, studies by various workers (Landsteiner and Levine, 1932; Todd, 1930, 1931, 1935; and unpublished data from this laboratory) on the cells of the chicken indicate that a very large number of cellular antigens is present in that species also, making more plausible the postulate that one or more genes on at least the majority of the chromosomes of a species may have effects on the cellular antigens.

Indeed, if a gene is a chemical entity, and if each gene is present in every cell of the body—at least in all those which have a nucleus—it might well be argued that every gene should be represented by an antigen in the red blood cells of birds, since they possess a nucleus. On such a basis the upper limit of the number of antigens in a given kind of cells would be the number of genes which they contained. However, it is conceivable that many more genes may have antigenic effects on the cells than can be readily detected. That is, on the assumption that the immunological reactions are surface phenomena, there may be many antigens which are not expressed at the surface of the cells, and hence would not react with an immune serum. What appear to be examples of this kind are assumed to exist in bacteria (Topley, 1933). Also, in human cells, a property (T) is detectable only after the action of environmental influence on the corpuscles (Friedenreich, 1938).

Several pertinent questions might be raised at this point. For example, to what extent may antigenic differences between individuals in either Pearlnecks or Ring

doves have influenced these findings? That is, do any Ring doves possess cellular characters which are supposedly peculiar to Pearlneck? At present, this question may be answered in the negative. More than five hundred Ring doves from various sources have been tested at one time or another over the past ten years, without a suggestion that any one of these possessed even one of the specific Pearlneck characters.

B. *Relationships of antigens of one species to those of two others.* It is of interest also to inquire whether any of the cellular antigens which distinguish Pearlneck from Ring dove are found in any other species. Should they be, the various single characters of Pearlneck would serve as "testers" to determine what combination of these was shared between Pearlneck and a third species, to the mutual exclusion of Ring dove. In such comparisons, a third species, the Senegal (*St. senegalensis*), has been of special interest because not only do its cells appear to contain nearly all the substances *common* to Pearlneck and Ring dove but, in addition, its corpuscles possess at least a part of each of the cellular characters peculiar to Pearlneck, not in Ring dove (Irwin and Cole, 1940). From such results it may be stated that at least to this extent the cells of Senegal may be differentiated from those of Ring dove.

If Senegal cells contain *all* the specific parts of Pearlneck cells, and nearly all those common to Pearlneck and Ring dove as well, it would be practically impossible to distinguish Pearlneck cells from those of Senegal. However, reciprocal comparisons of the corpuscles of these two species (Pearlneck and Senegal) have shown that a distinction between them may be as readily accomplished as between either and Ring dove. Clearly, then, not all the specific components of Pearlneck are contained *in toto* in Senegal. The actual tests (Irwin and Cole, 1940) have revealed that Senegal shares with Pearlneck all of 7 cellular antigens (d–1, d–2, d–3, d–4, d–5, d–7 and d–9), but only a part of three others (d–6, d–10 and d–11). More

recently another Pearlneck character (d-12) has been recognized, which is not shared with Senegal *in toto*, if at all.

The relationships so far described between the specific cellular characters of Pearlneck and the Senegal complex are based entirely on findings by immunological technics. On genetic grounds, if Pearlneck differs from Senegal mainly if not entirely in three cellular antigens (d-6, d-10 and d-11), these should segregate in Mendelian fashion in backcross offspring, following the cross of Pearlneck and Senegal. At the time such tests were made, it was recognized that Pearlneck shared with Senegal a part of d-6 and d-11, and in the first backcross generation (to Senegal) there appeared the four kinds of individuals expected; viz., those with both d-6 and d-11, those with either d-6 or d-11 and those with neither (Irwin and Cole, 1940). (There was also a third character segregating, which probably was d-10, although the differentiation of d-6 and d-10 is not always possible.) Thus the genetic segregation in the backcross offspring entirely confirms the relationships proposed by the use of direct immunological tests.

This resemblance but not identity of heritable antigens in Senegal to the three single characters of Pearlneck (d-6, d-10 and d-11) may be explained in either of two ways, or by a combination of these. (1) If each of these three antigenic characters of Pearlneck is produced by two or more genes on each of the chromosomes, there may be genes in Senegal identical with some, but not with all, of the respective genes of Pearlneck producing the three characters. On this explanation only that part of each of these three antigens of Pearlneck would be shared with Senegal, for which Senegal possessed homologous genes. For example, if the d-11 character of Pearlneck were the result of the joint action of but two linked genes, Senegal might have a gene homologous to one of these. That species would thereby have a part, but only a part, of the Pearlneck character d-11. (2) On the other hand,

if each of these three characters peculiar to Pearlneck were the result of the action of a single gene, the only reasonable conclusion would be that in Senegal there were genes producing similar but not identical chemical effects. The genes themselves in each of the two species therefore would presumably be related, but not identical, in their own chemical constitutions. Several examples of this kind of relationship appear to obtain between the known antigens of human blood cells, supposedly produced by single genes, and those of the anthropoid apes and lower monkeys (Landsteiner and Miller, 1925a, 1925b; Landsteiner and Wiener, 1937; Wiener, 1938, 1943).

It may be concluded from the above evidence that the biochemical relationships between Pearlneck and Senegal are much closer than those between Pearlneck and Ring dove. It was stated earlier that approximately 10 of the probable 30 pairs of chromosomes of Pearlneck carry one or more genes that produce cellular antigens which differentiate Pearlneck and Ring dove. On this same basis, only three, or possibly four, chromosomes of Pearlneck carry genes which distinguish its blood cells from those of Senegal.

These findings suggest still another means of assaying relationships between Pearlneck and Senegal in contrast to Ring dove. The experimental results have shown that Pearlneck shares *in toto* with Senegal seven of the ten or so cellular components which distinguish Pearlneck from Ring dove. Should the cellular characters which differentiate Senegal from Ring dove be obtained in unit form, will there be seven of these characters indistinguishable from and therefore supposedly identical with the seven known characters of Pearlneck? Or will they exist in different combinations in Senegal, as would be expected if in that species there were different linkage relationships of the causative genes? Only a partial answer to these questions can be given at present. From unpublished data, it does appear that two specific antigens (d–3, d–4) of Pearlneck are also units in Senegal. However, at

least one antigen of Pearlneck (d–1) appears in Senegal to be inherited as two substances rather than as one, indicating that in Pearlneck this character is caused by the joint action of at least two linked genes. Studies of the reciprocal relationships of the unit antigens of Pearlneck and Senegal, respectively, not in Ring dove, are being continued and will be reported in detail elsewhere.

These results and those from other comparable experiments (Irwin *et al.*, 1936; Irwin, 1938) allow the general statement that the genetic relationships of the blood cells of various species may be determined by appropriate immunological tests. Naturally, these will not reveal the number of antigenic characters differentiating one species from another. In this connection, comparisons of eleven species of the genus Columba (Irwin and Cumley, 1943) have shown that the antigens peculiar to one species in comparison with another were usually shared, at least in part, with several other species. Also, for certain species in relation to others it was found that a given species might share (a) a complex of cellular characters with one species, (b) the same complex or the same complex plus additional antigens with another species, (c) all the components of the second species plus others with a third, etc. Each of the species studied, in its relationships to the others, appears definitely to be an entity. The cellular characters of each species interlock in intricate but somewhat dissimilar patterns with those of the others. Despite differences in interlocking relationships towards other species which might account in part for the differentiation between two species, it appears possible for a species—but not necessarily all species—to contain antigens (therefore genes) which were not found in any others of those tested.

Antigens in the Serum

The question naturally arises now as to whether the serums of these species have comparable specific antigens and, if so, whether the same genes have effects in both

serum and cells. As is well known, a considerable body of experimental data has accumulated on the interrelationships of the serum antigens of many species, following the classical researches of Nuttall (1904). Although it undoubtedly has been tacitly assumed by many of the investigators that these experimental studies revealed some sort of genetic relationships between the groups under comparison, only recently has definite evidence been obtained that the species-specific antigens of the serum are gene-determined.

As was found in attempts in our laboratory to differentiate the cells of closely related species of birds, so also was it found to be impossible to differentiate the serums of these same species by the use of untreated antiserums. (The antiserum is diluted in determining its agglutination titer, whereas in the usual tests to determine the precipitation titer of an antiserum, the antigen is diluted, and the antiserum is used undiluted or at a constant dilution. To many workers it seems illogical to apply the same reasoning to the results obtained from such tests as can be used if the antiserum, not the antigen, is diluted.) But if an antiserum prepared in a rabbit against the serum antigens of one species was absorbed by the serum of another species, a "reagent" was prepared which would react with the homologous, but not with the absorbing serum. Thus, in our experiments the differentiation of the two kinds of serums has been on the basis of the presence or absence of a reaction. Following the same terminology used for the relationships of the cellular characters of two species, the reactive components of the homologous serum with the absorbed fluids have been termed "species-specific," and those for which antibodies have been removed in the absorption have been called the "common" constituents of the serum of one species in relation to the other. This terminology has no implications as to the structure of the serum antigens, which presumably are proteins.

A segregation of the serum antigens specific to Pearl-neck, comparable to that noted in cellular antigens, has been observed in the progeny of successive backcrosses to Ring dove. Although these specific serum antigens have not yet been obtained in unit form, as have the cellular antigens, it is reasonably certain that there are three or more distinct serum antigens peculiar to Pearlneck (Cumley and Irwin, 1942, and unpublished data), whose distribution in the backcross families simulates that expected if each were the result of the action of one or more genes on a particular chromosome. It is significant that there was independent segregation of the antigens of the cells and serum, so that a single backcross bird might have Pearlneck specific antigens in either cells, serum or in both, while both kinds of antigens might be entirely lacking in another. Therefore the possibility is eliminated that the antigens of the serum are in the nature of disintegration products of the cells, as has been noted in the serums of some humans whose cells carry **A** or **B**, or both. (An extensive analysis of the proportions of individuals carrying cellular-like substances in their serums has been reported by Aubert *et al.*, 1942.)

Corroborative evidence of segregation of the species-specific components of the serum, and their probable genetic independence from the specific cellular components, has been obtained in backcross offspring of three other species-hybrids (Cumley, Irwin and Cole, 1941; Cumley *et al.*, 1943; Irwin and Cumley, 1942). The present evidence indicates that, for each of the various species whose species-specific antigens have segregated in backcross progeny, between three to five serum antigens distinguish the serum of the one species from that of the other. The implication follows that as many chromosomes were involved as there were antigens peculiar to a species. These findings make reasonable the conclusion that all the biochemical constituents of the serum, not only those which are species-specific, of these and other species are gene-determined.

The two kinds of antigens of the blood of Pearlneck may now be used together to give a more accurate analysis of the biochemical relationship of this species to Ring dove than is possible with either kind of antigen alone. In terms of chromosomes, then, it appears that the Pearlneck has ten chromosomes each with one or more genes with effects peculiar to itself on the cellular antigens and, seemingly, a minimum of three other chromosomes carrying genes which produce specific antigens of the serum. Thus, out of a total of approximately 30 pairs of chromosomes, Pearlneck has 13 or more with genes affecting specific antigens in either serum or cells. That is, slightly less than half the chromosome complex of Pearl-

Diagram of the species-specific and common constituents of the blood of Pearlneck, in comparison with those of Ringdove.

Specific cellular components   Specific serum components   Common components in both serum and cells   Pearlneck
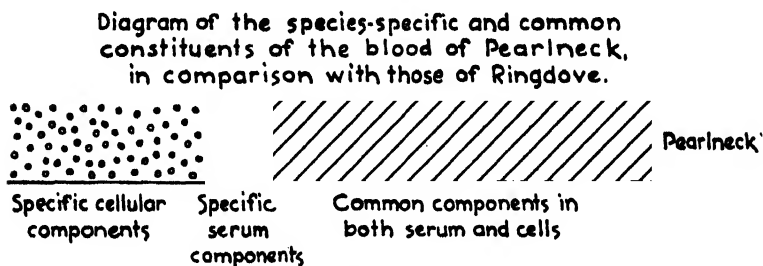
Fig. 1.

neck—but not necessarily the same proportion of the chromatin material—carries genes which differentiate its blood from that of Ring dove. A diagrammatic sketch of the summation of these different kinds of antigens of Pearlneck in comparison with Ring dove is given in Fig. 1.

Furthermore, as stated earlier, Pearlneck is more closely related to Senegal than to Ring dove, on the basis of cellular characters, differing from that species in antigens produced by genes on only three or four of the probable ten chromosomes which set it apart from Ring dove. Indeed, as explained above, a part of the effects of the genes on these three or so chromosomes of Pearlneck, carrying genes producing antigens not in Ring dove, actually are shared with Senegal, implying linkage of genes producing *common* and *specific* effects, respectively. On the other hand, in antigens of the serum Pearlneck appears

to differ from Senegal in about the same number (three), but possibly not in the same serum antigens, as distinguish it from Ring dove (unpublished data). Only six or eight of the 30 pairs of chromosomes of Pearlneck carry genes whose effects on either cells or serum distinguish its blood from that of Senegal.

Thus it may be concluded from these findings that the genes which produce the "major" biochemical differences between these species are located on a relatively small proportion (less than half) of the chromosomes of the respective species, rather than being scattered at random over most of the chromosomes. All data of this laboratory, published and unpublished, support this conclusion. If each of the antigens specific to one species is the cumulative effect of many "small" genes on a particular chromosome, the overall picture of gene-differences between species might, with some modification, fit in with current genetic theory of such differences, as discussed by Muller (1940). But if each such antigen were produced by the action of a single gene, or by a few linked genes, it hardly seems that these data would fit the theory that the differences between species depend upon multiple genes, having individually small effects.

At present no definite statement can be made as to the probable number of genes effecting any one of the species-specific antigens thus far identified. However, if any one of the cellular antigens which have been isolated as presumed units (of Pearlneck as contrasted with either Ring dove or Senegal, or of *Columba guinea* as compared to *C. livia*) were produced by the cumulative action of many genes, one might well anticipate an occasional further fractionation of the antigen as a result of crossing over. Naturally, the linkage relationships of these hypothetical small genes in the one species as compared with those in the other would determine whether there was any interference with normal synapsis and subsequent crossing over. No information is at present available on this subject. As stated earlier, the criterion for each unit

antigen has been approximate equality of birds with and without the component in the backcross offspring of an individual carrying the character. Routine tests have nearly always been made of the cells of backcross progeny carrying the antigen to see if they possessed all or only a part of the cellular component found in the parent. At present, only three possible instances of fractionation of any of these antigens, presumably following crossing over, have been noted; two of the d–1 and one of the d–4 character. No offspring were obtained from these three individuals so genetic verification of the probable fractionation of the respective antigens is not available. From the evidence at hand, it appears that crossing over involving the chromosomes carrying genes for the species-specific characters has been very infrequent in the backcrosses, if it has occurred at all. But this evidence does not permit any statement of the kind of effects—whether small or large—of the causative genes.

In each of the four kinds of species crosses in which a segregation of antigens of the serum and cells has been demonstrated, the two kinds of antigens have separated independently. Although the possibility of a loose linkage of the causative genes can not be entirely eliminated, it seems probable that the species-specific antigens of the cells and serum are produced by genes on independent chromosomes. One might wonder whether there is a mutual exclusion of linkage of genes affecting the species-specific antigens of the serum and cells, respectively, or whether the independence observed in the four different species crosses was only fortuitous. However, at present there is no known reason why there may not be linkage of genes with effects on both serum and cells. There may well be such linkage between the genes affecting the *common* components of both serum and cells in the species already tested.

Just why there should be a smaller number of chromosomes in Pearlneck, in relation to Ring dove, which carry genes with species-specific effects on the serum, as com-

pared with the number whose genes affect the specific cellular antigens, is not at present answerable. One explanation might well be that many of the antigenic components of the cells are other than proteins (*i.e.*, they may be substances with antigenic activity because of linkage to proteins, *i.e.*, haptens), whereas those of the serum are probably proteins. On this basis, it would appear that the proteins are somewhat the more conservative genetic characters. But Pearlneck differs from Senegal in three or four cellular components and in about the same number of serum antigens, so that if there is a difference in the kind of antigens of serum and cells, those of the serum need not always be the more conservative.

A summary of all the comparisons which have been made between the serums of various species of animals, and between extracts or definite proteins of species of plants, would undoubtedly show that serum differences, presumably protein in nature, are one of the decisive tests in the differentiation of species. In other words, it is probable that all or nearly all species have protein specificity. Are protein differences, then, a line of demarcation only between species, or may there be such differences between individuals within a species?

Some months ago it was noted in our laboratory that the serums of hybrids between the pigeon (*Columba livia*) and Ring dove did not react alike following their use in absorptions of pigeon antiserum (unpublished data). Further tests lead to the conclusion that there were definite antigenic differences between the serums of individual hybrids, implying heterozygosity of the causative genes in the parent pigeons. If this be the correct explanation of the findings and their implications, differences definitely are to be found among the serums of individuals of this species. The small amount of serum that can be obtained from a single pigeon, however, almost precludes extensive tests of the correctness of this conclusion.

Following these findings, attempts were made to determine if there were individual differences in human serum. Definite evidence of such differences has been obtained very recently (Cumley and Irwin, 1943) in that the serums of some humans proved to be distinguishable from those of others. At the present writing, it must be admitted that there is a possibility that the antigenic substances in the serum were metabolic products of one kind or another, although it is much more probable that they represent definitive antigens. Should future tests establish that there are antigens in human serum which obey Mendelian principles, a new class of substances would become available for research in human genetics.

If these differences are genetic, and especially if it should be proved that they represent differences in proteins between individuals *within* a species—whether in humans or any other species—it obviously would no longer be correct to state that protein differences exist only *between* species. Furthermore, it appears reasonable to propose that any changes in the chromosomal material which affect the constitution of the proteins of a species would be a probable point of departure of incipient species. In any event, studies of the nature of the changes in chromosomal material required to affect the constitution of the proteins would be both interesting and valuable.

Because serum is made up of different kinds of proteins, one of the lines of work for the future is to determine whether the differences in serum antigens between species and between individuals are proteins, and what kind of proteins, or simply components linked to proteins. The immunological specificities of the types of pneumococci presented a problem to the biochemist, which, although brilliantly attacked, is still not completely solved. (Parallel studies made by other workers with various species of bacteria have put new life into research in bacteriology.) So also do the antigens of the cells and serum (and various body tissues as well), whether inter-

or intra-specific, challenge the biochemist for the analyses of the. nature of the components. What is required for the future are the combined efforts of three branches of science—chemistry, genetics and immunology—each being dependent upon the other two for what humans are pleased to call the "final answers."

In conclusion, perhaps a bit of speculation on another subject may be permitted. Only within the last two decades have experimental findings allowed the immunologists to break away from the belief that only proteins were concerned in immunological reactions. By an ingenious technic, to which again we are indebted to Landsteiner, it has been demonstrated that relatively simple chemical substances, if attached to proteins, may be antigenic. Probably most geneticists at the moment are unable to think in terms of the constitution of genes as being other than protein. Leaning heavily upon findings in the field of immunochemistry, the suggestion is made that many genes may be less complex in nature than proteins— such as carbohydrates—but may owe their biological activities to their linkage to proteins. (It is probable that other biologists have somewhat the same concept of the possible nature of genes. For example, one of us in conversation with Professor C. E. Allen found that the above suggestion coincided very closely with one which he had held.) If the cellular antigens are more or less direct products of their causative genes, a chemical analysis of the nature of these antigens should be one of the most promising approaches to a knowledge of the chemistry of the gene itself.

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SEROLOGY AND ANIMAL SYSTEMATICS[1]

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INTRODUCTION

DISCOVERED by Rudolf Kraus in 1897, the precipitin reaction was first extensively applied to the problems of animal systematics by Nuttall, whose pioneer work in systematic serology is known, but not too well known, to most zoologists. Kraus (1897) at first understood the reaction to be absolutely specific, *i.e.*, each antiserum was capable of reacting only with the particular antigens used in its formation. Obviously such specific antisera can be used for *differentiating* antigens or the species characterized by them, but not for *classifying* them. So convinced were the early immunologists that the precipitin reaction was absolutely specific and therefore a grand device for identifying antigens, such as those present in blood stains, that Tchistovitch (1899) even claimed that antihorse serum would not react with ass serum, two closely related species being represented in these tests. Later investigators have had more difficulty in distinguishing between them, a distinction which can be made if the technique is more quantitative than that used by Tchistovitch.

The first report proving that the precipitin reaction was not absolutely specific was that of Bordet (1899) and the entire series of applications of the precipitin reaction to animal systematics rests on his discovery that an anti-chicken serum produced in a rabbit reacted most strongly with chicken serum *but also reacted, though much less strongly, with pigeon serum!* This was indeed the origin of the principle of quantitative specificity which now con-

stitutes an essential part of the foundations of systematic serology. This principle states that each antiserum reacts most strongly with the particular antigens used in its formation (homologous reactions) and less strongly with other antigens (heterologous reactions) under comparable conditions. It was Nuttall's great contribution to have demonstrated the truth of this principle in the field of animal systematics and to have shown furthermore that the relative intensities of precipitin reactions did parallel the systematic positions of the species whose antigens were tested.

As a matter of fact Nuttall's first concern with the precipitin reaction was with its medico-legal application to the problem of identifying blood stains, but he quickly saw its possibilities as an aid to taxonomy. Thus he reported (1901a) on the results of testing several mammalian antisera, some of which showed weak cross reactions, and concluded: We have in this test the most delicate means hitherto discovered of detecting and differentiating bloods, and consequently we may hope that it will be put to forensic use.''

But his next report (1901b), giving the results of testing 140 bloods and stating his intention of collecting and testing as wide a variety of bloods as possible, led him to say, ''It seems certain that interesting results from the point of view of zoological classification will thus be brought to light.'' In fact, with each succeeding report his enthusiasm and faith in the precipitin reaction increased and his statements became more positive. From his next report (1901c) we quote: ''The above experiments, which are being prosecuted on a large scale, the attempt being made to obtain a variety of antisera, indicate with certainty that we possess in this test a most valuable aid in the study of classification of animals.''

In a subsequent progress report (1902) Nuttall described an improved technique of measuring the amounts of precipitate formed, gave some actual measurements which accord remarkably well with the systematic posi-

tions of the species tested, and made the following most interesting and understanding statements: "I do not wish these numbers to be taken as final, nevertheless they show the essential correctness of the previous crude results. To obtain a constant it will be necessary to make repeated tests with the bloods of each species and with different antiserums of one kind, making the tests with different dilutions and different proportions of antiserum. I am inclined to believe that with care we shall perhaps be able to 'measure species' by this method, for it appears from the above results that there are measurable differences in the reactions obtained with related bloods, in other words, determinable degrees of blood relationship which we may be able to formulate."

This is a most remarkable statement to have been made over forty years ago, showing that Nuttall had a basically correct understanding of the possibilities, both practical and theoretical, of quantitative precipitin testing. To "measure species" would indeed be a contribution to animal systematics of the greatest value, for taxonomy like all other branches of zoology must become more objective and quantitative to progress. But unfortunately Nuttall did not practise what he preached regarding the use of varying proportions of antisera and antigens and consequently he could not measure species—no, not even did he correctly measure the relative intensities of the reactions he performed. In spite of his 16,000 tests and more, summarized in his classic of 1904, he failed to use anywhere an adequate or good precipitin technique. In systematic serology useful ends can not be obtained without adequate means and to understand the sources of error in Nuttall's comparisons as well as those of many later investigators we must now turn briefly to matters of technique.

Some recent publications (Boyden, 1942; Boyden and De Falco, 1943, in press) have described the principal techniques used in precipitin testing as applied to animal systematics, and pointed out their chief sources of error.

The following account is, therefore, very brief. The two principal techniques used in precipitin testing and animal systematics are (1) the flocculation test and (2) the ring test. The former usually involves the mixture of constant amounts of antiserum with decreasing amounts of antigen, followed by the incubation of the reagents and the recording of the highest dilution of antigen in which appears a settled precipitate, or a greater turbidity than shown by the control tubes. Usually only the endpoint or "titer" is recorded, and the actual amounts of precipitate are ignored. Nuttall, however, used only a single, and *unknown*, amount of antigen, and crudely estimated, or later "carefully measured" the volume of precipitate obtained from that proportion of antigen and antibody. I say Nuttall used an unknown amount of antigen because the choice of a one to one-hundred or one to two-hundred dilution of any blood serum or filter paper extract of dried blood is really in a quantitative sense unknown as to its antigen concentration. But the flocculation test can be quantitatively performed with known amounts of antigen and antiserum, where the volumes of precipitate are measured after centrifuging (Boyden and Baier, 1929; Baier, 1933); where the nitrogen contents of washed precipitates are determined (Wu *et al.*, 1928; Heidelberger and Kendall, 1929); or where the turbidities developed may be accurately measured by such an instrument as the photronreflectometer (Libby, 1938). These are truly quantitative methods and are therefore superior to the earlier flocculation techniques. The ring test first developed by Ascoli (1902) involves a careful layering of the antiserum under successive dilutions of the antigen and it is usually recorded in terms of the titer or endpoint, *i.e.*, the highest dilution of antigen which shows a distinct layer of precipitate at the zone of contact of antigen and antiserum. Ring test endpoints should be sharper than the corresponding flocculation end points, and in this respect the ring test is superior to the crude flocculation test.

These essential matters of technique which must be understood before we may successfully evaluate the results of precipitin testing in the field of animal systematics may best be explained by reference to Fig. 1. Here are shown the data obtained from ring tests, and from photron-'er measurements of the turbidities resulting
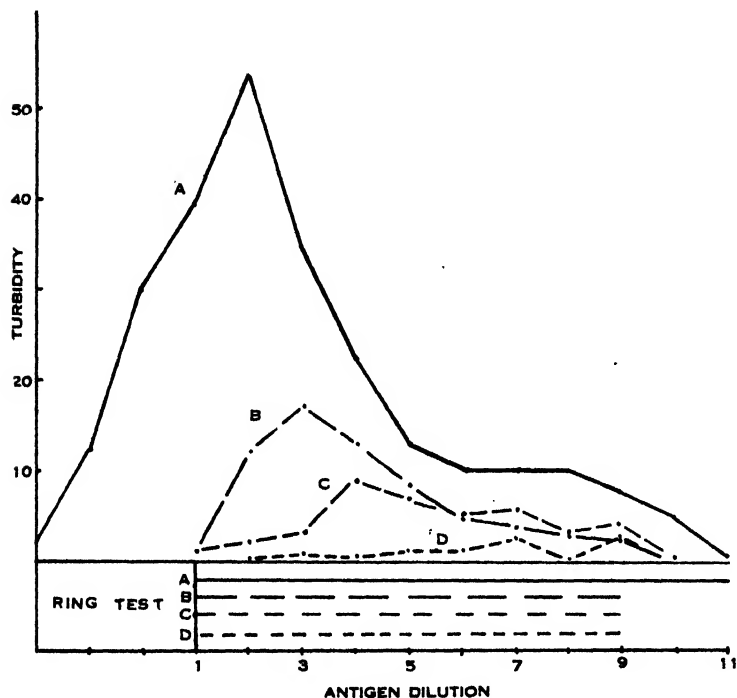
FIG. 1. A graph to show the results of testing an anti-*Callinectes sapidus* serum (140b) with standard dilutions of the sera of *Callinectes sapidus* (line A), *Carcinus maenas* (line B), *Cancer borealis* (line C), and *Menippe mercenaria* (line D). The upper part of the figure gives the photron'er turbidities along the ordinate correlated with the antigen dilutions along the abscissa. Dilution "1" is one part of haemocyanin in 500 parts of buffered saline and each succeeding dilution has half the concentration of its predecessor.

The lower part of the figure shows the corresponding ring test titers for the same species and antigen dilutions.

from the interaction of an anti-*Callinectes sapidus* antiserum obtained from a rabbit, tested with comparable amounts of the sera of *Callinectes sapidus* (A), *Carcinus maenas* (B), *Cancer borealis* (C) and *Menippe mer-*

cenaria (D). The lower part of the figure gives the ring test results; the curves above are the turbidities as measured by the photron'er. Galvanometer readings of the photron'er appear on the ordinates, the antigen dilutions are plotted along the abscissa. Dilution "1" represents one part of haemocyanin to 500 parts of buffered saline and each succeeding dilution is one half the concentration of its predecessor. Thus dilution "2" is 1:1000 and "3" is 1:2000, etc.

Now the salient points obtainable from a study of Fig. 1 are these:

(1) The homologous reactions exceed all others to a considerable degree.

(2) The relative intensities of the reaction accord well with the systematic positions of the species.

(3) The photron'er comparisons give readings throughout the entire reaction range of each test whereas,

(4) The ring test records only the endpoints or titers and is therefore generally a less adequate and less discriminating method for the comparison of related bloods.

(5) The photron'er tests show the real nature of the precipitin reaction as a phenomenon of optimal proportions, and compare the antigens throughout their entire reaction range with a given antiserum, whereas the ring test records only the titer or relative sensitivity of an antiserum when tested with different antigens.

Now this comparison of a truly quantitative flocculation technique with the widely used ring test gives all the advantages to the former. Actually there is one more respect in which the photron'er comparisons surpass the ring test, *viz.*, the former *requires no independent proof of equivalence in the amounts of antigen used* provided the curves are complete, touching or nearly approaching the value of zero turbidity at each end. For ring test titers to be comparable, when an antiserum is tested with a variety of antigens, the antigens compared must be tested in equivalent amounts, for the position of the endpoints is dependent on the relative sensitivities of an antiserum *measured by* the smallest concentrations of the various antigens capable of reacting with it. If these concentrations of antigen are unknown or not equivalent the whole basis of comparison becomes invalid.

On the other hand, provided the curves are complete, the photron'er gives the relative reaction intensities of an antiserum with a variety of antigens and no more reaction is possible. In view of the fact that serological equivalence is usually difficult to establish, it is obvious that the photron'er's inherent advantages justify our inclusion of no further ring test results in this report.

One more consideration requires further explanation. The fact that the precipitin reaction is a phenomenon of optimal proportions, so nicely illustrated in Fig. 1, makes it obvious at once that the proper basis of comparison in quantitative flocculation tests is the curve, the whole curve and nothing but the curve. It is clear that any lesser basis of comparison could fall into serious error. Thus there is no single antigen dilution which falls on the same relative position to the whole curve for the different sera tested. Therefore, since the whole reaction range of each antiserum and each antigen must be determined, the natural procedure which we have adopted is to compare the relative areas of whole curves. No other basis for comparison is adequate, and here is where Nuttall made a great mistake. He "carefully measured" the volume of precipitate at some one unknown point of the curve of optimal proportions and thus he and others too have given us data of unknown and unproven comparability. Far from "measuring species" they failed to adequately measure the relative intensities of the precipitin tests made by them.

With this brief discussion of essentials regarding technique we should be able to properly evaluate the data already obtained and those presented in this and subsequent reports. But now what of the general theory of systematic serology? Where does systematic serology stand in relation to zoology as a whole? The fundamental principles of systematic serology are these:

(1) The antigenic composition of animals is an important part of their essential natures and must be considered in any sound natural system of classification.

(2) Protein antigens are conservative hereditary traits.

(3) Good precipitin techniques are well adapted to reveal the relative degrees of biochemical similarity of protein antigens.

With regard to these principles the first can scarcely be disputed. It is generally admitted by taxonomists that the more we know of animal nature, the more "natural" our classifications may become. From the standpoint of objectivity alone, biochemical comparisons outrank most morphological description. Paleontologists, from obvious limitations, may be restricted to morphological comparisons and fragmentary ones at that, but surely no one will recommend that taxonomists ignore the biochemical composition of recent organisms which is as essential a part of their natures as any morphological features. Actually biochemical comparisons fall within the province of "morphology," though in this case it is a matter of the structure and configuration of atoms and molecules.

The second principle is a short statement of the essential truth that proteins are at least as constant and characteristic of animal species as their morphological features and are fundamentally determined in their expression by a genetic mechanism. It is true that the specific nature of an organism is determined primarily by inheritance and this inheritance applies to proteins as well as to gross morphological features. Recently the qualities of serum proteins have been found to be inherited. Thus Boyden (1942 and earlier) finds that the serum of the mule is intermediate between that of horse and ass, and Cumley, Irwin and Cole (1941), and Cumley and Irwin (1942) report the typical inheritance of serum protein differences and resemblances in their dove hybrids. So far as is known at present the quality of the serum proteins is not affected by changes in the environment and thus serum protein antigens bear the characteristics of conservative inherited traits.

Finally, the relative intensity of precipitin reactions, when adequate techniques are employed, is a measure

of the serological and chemical similarity of the antigens tested and this principle has been abundantly proved by the investigations of Landsteiner and his associates as summarized in Landsteiner (1936) and by the investigations of Marrack and others reviewed in Marrack (1938). Some of the comparisons between antigenic constitution and serologic specificity reported by these workers were based on relatively crude techniques and further studies of a more truly quantitative nature are needed but there is no reason to doubt the general proportionality between chemical nature and serologic specificity.

The theory of systematic serology is sound, but there have been too few adequate applications of good precipitin techniques to problems of animal systematics. We are at present far from the goal of systematic serology but the potentialities for useful contributions to the solution of taxonomic problems have become clearer in recent years. As an example of such potentialities we present briefly the results of recent studies on Crustacean relationships as obtained by the new photron'er technique. These will be briefly compared with the results of photron'er studies on other groups of animals, which have been conducted in our laboratory.

PHOTRON'ER COMPARISONS OF THE SERA OF COMMON CRUSTACEA AND THEIR INTERPRETATION

These studies came chiefly as a result of the interest, aid and encouragement of Dr. Waldo L. Schmitt, of the U. S. National Museum. It is a pleasure to acknowledge my debt to him for the loan and gift of specimens, for aid in their collection, for the identification of species and for advice regarding the problems of Crustacean systematics. I am indebted also to Dr. A. C. Redfield, of Harvard University, for first pointing out the fact that Crustacea are favorable species for serological studies on account of the high proportion of a single protein, haemocyanin, in their sera. Actually, according to Allison and Cole (1940), haemocyanin is the only protein present in the sera of common Crustacea.

The antigens were collected over a period of years involving four trips to the Tortugas Laboratory of the Carnegie Institution, and one trip to the United States Bureau of Fisheries Laboratory at Beaufort, N. C., one to the Mt. Desert Island Biological Laboratory at Salsbury Cove, Me., and one trip to the Laboratory of the Marine Biological Association at Plymouth, England. The blood was obtained from the various species of crabs by the removal of a fifth pereiopod, and from the lobsters and crawfish by a ventral incision through the membranes in the region of junction of cephalothorax and abdomen. For large animals the blood from single specimens was sometimes kept as separate samples; in all other cases pooled bloods were obtained. These blood samples were allowed to clot, stored for a few hours in the refrigerator, and the serum was poured off. These sera usually remained in an oxidized state and were so filtered through Seitz filters and stored sterile in small vials. They have been kept in the icebox since their arrival in the Rutgers laboratory.

The antisera were, in most cases, prepared in rabbits in accordance with a standard injection procedure, *i.e.*, the initial intravenous dose was 5 mgms of protein per kg of body weight of the rabbit, followed by three other doses on alternate days, each of which contained twice the protein content of the preceding dose. Seven to ten days after the last injection the rabbits were bled completely, under ether anesthesia, by cardiac puncture. The rabbit's blood was allowed to clot with minimum disturbance, and the serum was collected as formed. The antisera so obtained were filtered sterile through Seitz filters and stored in small vials in the refrigerator.

The photron'er tests were performed as briefly described by Boyden (1942) and as described in detail by Boyden and De Falco (1943, in press). The data obtained as a result of testing fourteen antisera with a variety of Crustacean sera are shown in Figs. 1, 2 and 3 and summarized in Tables I and II.

Fig. 1 (upper part) shows the results of testing an antiblue crab serum (I40b) with the sera of the blue crab, *Callinectes sapidus* (line A); the green crab, *Carcinus maenas* (line B); the Jonah crab, *Cancer borealis* (line C), and the stone crab, *Menippe mercenaria* (line D). The relative areas under the curves are as follows: A,
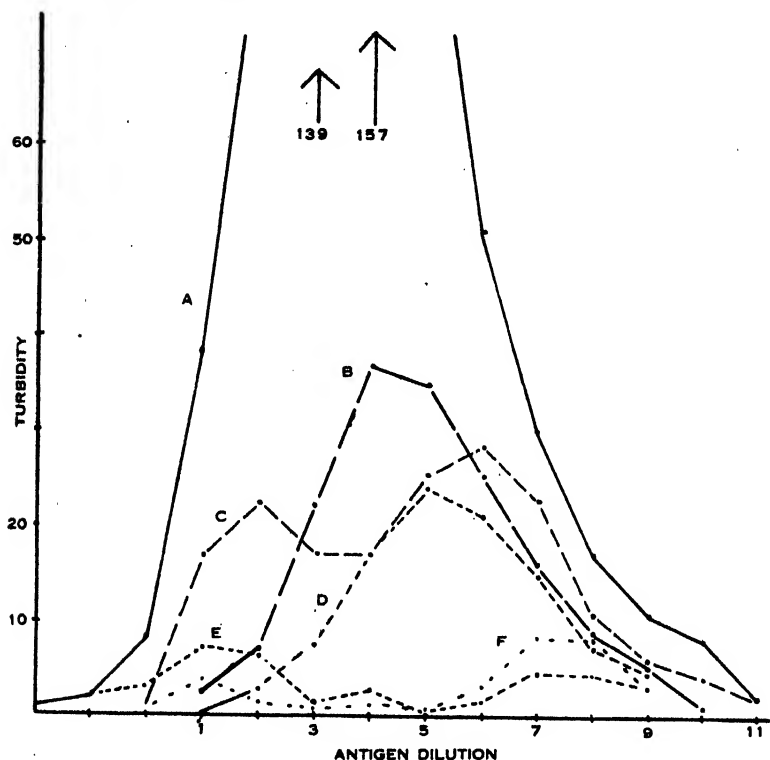
FIG. 2. A graph to show the turbidities resulting from the titration of a powerful anti-Menippe serum (I63) tested against the following antigens: *Menippe mercenaria* (A), *Geryon quinquedens* (B), *Callinectes sapidus* (C), *Cancer borealis* (D), *Ocypode albicans* (E), and *Maia squinado* (F). The corresponding areas are given in Table 1. This antiserum as diluted 1 plus 2 parts of saline gave a homologous area of 645 units. It could have been further diluted but was used as the 1 plus 2 dilution in order to obtain significant measurements of the more distantly related families.

homologous reaction, 100 per cent.; B, 26 per cent.; C, 16 per cent. and D, 4 per cent.

Fig. 1 (lower part) shows the results of the ring tests on the same species. Here the corresponding titers are

100 per cent., 25 per cent., 25 per cent. and 25 per cent., respectively.

Obviously the differentiation of the species tested is better shown by the photron'er results than by the ring test results.   The previous discussion of the two techniques should explain why the photron'er is capable of more adequate comparisons than the ring test.   Furthermore, the photron'er data for these species accord pretty well with their systematic positions whereas the ring test results do not.   Thus Callinectes and Carcinus are genera of one family, the Portunidae; whereas Cancer and Menippe belong to the different families, Cancridae and Xanthidae, respectively.

Fig. 2 shows the reactions of a more powerful antiserum (I63—anti-*Menippe mercenaria*).   It was tested with the sera of Menippe mercenaria (A); *Geryon quinquedens* (B); *Callinectes sapidus* (C); *Cancer borealis* (D); *Ocypode albicans* (E), and *Maia squinado* (F).   The range of this antiserum was great enough to include all the families of crabs tested; indeed, from the mountain peak of 157 units turbidity, one should be able to see even as far as the spider crab, Maia, way over on the horizon. As far as can be stated at present, the relative curve areas constitute a fair approximation to the systematic positions of these species, though powerful antisera are generally less specific than antisera of moderate strength and the area of the Callinectes curve is too large in comparison with the reciprocal tests given in Table I.   We have shown (Boyden and De Falco, 1943, in press) how powerful antisera may be made more specific and more discriminating by dilution, and thus how antisera of different original grades of specificity may be standardized.

The curves shown in Figs. 1 and 2 are typical of a large number obtained in the study of other animal groups besides the Crustacea such as Insecta, Pisces, Aves and Mammalia.   Occasionally a bimodal curve is obtained or the curve may run a more rounded course, but these differences do not appear to justify the presentation of

more curves at this time.   Instead the data from all the Crustacean comparisons are summarized in the tables which follow.   In all cases the homologous area represents 100 per cent. and the heterologous per cent. values indicate the ratio of heterologous areas to the homologous area.

The data shown in Table I are incomplete since not all the antisera have been tested with a sufficient number of

<div align="center">TABLE I</div>

<div align="center">A COMPARISON OF THE SEROLOGICAL REACTIONS OF THE SERA OF COMMON CRUSTACEA</div>

Antiserum	Homologous antigen		Homarus america	Homarus vulgaru	allinectes sapidus	irclnus maenas	ancer borealis	ancer irroratu	ancer pagur	Menippe mere	Geryon quinquedens	Ocypode albica	Mala squinado
I42(1+2)	Homarus americanus	L3	100	54									
I40(1+0)	Callinectes sapidus	L4			100	26	16						
I47(1+0)	Callinectes sapidus	371A			100	44	14						
I54(1+1)	Callinectes sapidus	IIC38-1			100	17	17			7		3	1
I52(1+0)	Carcinus maenas	3			34	100	22			8			1
I48(1+1)	Cancer borealis	3b					100	58					
I51(1+1)	Cancer borealis	IIC1					100	59	41				
I60(1+1)	Cancer borealis	3d					100	29	19				
I61(1+1)	Cancer pagurus	39-1					41	33	100				
I62(1+0)	Cancer pagurus	39-2					55	51	100				
I49(1+0)	Menippe mercenaria	36-A				6	12			100	10		
I63(1+2)	Menippe mercenaria	36-A				28	16			100	25	6	5
I50(1+1)	Geryon quinquedens	36-1							19	24	100		
I64(1+1)	Geryon quinquedens	39-2			3					6	100		

antigens.   From the standpoint of animal systematics, however, they appear to have a special interest and since it may not be possible to complete these tests in the near future they are reported at the present time.

From the standpoint of animal systematics the data of Table I may best be presented as outlined below, for here the values are assembled so as to show most clearly their relation to the systematic categories concerned.

I. The relationships of the sera of species of the same genus.
  A. Cancer
      1. *Cancer borealis vs. C. pagurus* 41, 41, 19, 55 av. *39*
      2. *Cancer borealis vs. C. irroratus* 58, 59, 29 av. *49*
      3. *C. pagurus vs. C. irroratus*   33, 51 av. *42*
  B. Homarus
      1. *H. americanus vs. H. vulgaris* 54   *54*
                  Grand average *46*

II. The relationship of the sera of genera of the same family.
  A. Callinectes vs. Carcinus   26, 44, 17, 34 av. *30*

III. The relationship of the sera of different families of Brachyura.
  A. Portunidae vs. Cancridae   16, 14, 22, 13, 17 *av. 16*
  B. Portunidae vs. Xanthidae 4, 6, 8, 7, 6 (28) average
                           of 5 values *6*
  C. Portunidae vs. Goneplacidae   3, 3, *av. 3*
  D. Portunidae vs. Maiidae   1, 1, *av. 1*
  E. Cancridae vs. Xanthidae   12, 16, *av. 14*
  F. Cancridae vs. Goneplacidae   24, 4, *av. 14*
  G. Xanthidae vs. Goneplacidae 10, 25, 24, 6, *av. 16*
  H. Xanthidae vs. Ocypodidae   *6*
  I. Xanthidae vs. Maiidae   *5*

Now the values thus outlined must be considered tentative, for we have not yet reached the plane of attack on these problems at which we can guarantee the serological equivalence of the antisera and antigens tested. The variables and sources of error are discussed below. The data do give us a first quantitative approximation to the serological relationship of the species tested and they do appear generally to agree with their systematic positions. Of special interest in this connection may be the relatively sharp differentiation between the sera of *Homarus americanus* and *H. vulgaris*. These species are very similar morphologically and might even be mistaken for one species if their geographic distribution were continuous, but their sera are almost as different as those of the three species of Cancer are to each other. The conclusion appears to be justified that *H. americanus* and *H. vulgaris* are "good" species, and that they would probably not be able to hybridize even if their ranges were continuous. Another case of special interest may be the position of Geryon, representing the family Goneplacidae, in relation to the families Xanthidae, Cancridae and Portunidae.

According to Miss Rathbun (1918, p. 9) the family Gone-placidae "is most closely allied to the family Xanthidae" and our data confirm this conclusion but show in addition that the Goneplacidae are almost as close to the Cancridae as to the Xanthidae. More tests with a variety of anti-sera and antigens are needed in order to obtain a "constant," as Nuttall termed it, but the position of Geryon and the Goneplacidae in relation to the other families as
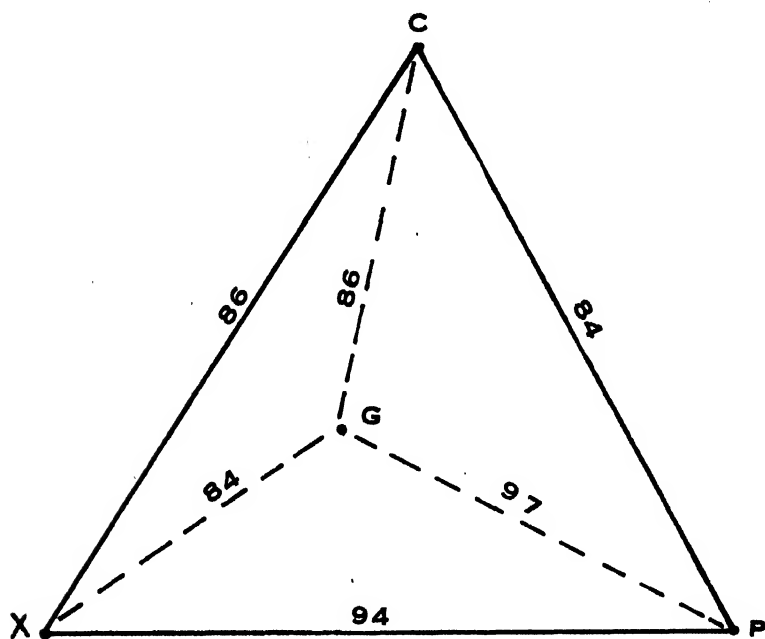
Fig. 3. A diagram to show the relative distances of 4 families of Crustacea from each other. The data are tentative inasmuch as the families have not yet had really adequate testing. The families concerned are: Cancridae (C), Portunidae (P), Xanthidae (X), and Goneplacidae (G). The latter family represented by Geryon is apparently closest to the Xanthidae represented by Menippe, a little less close to the Cancridae and considerably more distant from the Portunidae. Three dimensions would be required to express these relationships properly and the plane figure is really a projection of such three-dimensional distances onto a plane surface.

indicated by the present data is shown in Fig. 3. Actually three dimensions would be needed to express this relationship properly, but it can be done on a plane surface as shown here.

It must be understood that further tests are indicated not only to discover the normal values for these species but to gain a fairer representation of the families concerned. Thus Menippe has had to serve as the sole representative of the Xanthidae, and Geryon as the sole representative of the Goneplacidae. We hope later to have filled some of these gaps in the serological analysis of Crustacean systematics.

Meanwhile the variables, the sources of error, in the photron'er technique, must be continuously attacked. The photron'er appears to have solved that part of the problem of antigenic equivalence which is concerned with

TABLE II

THE EFFECTS OF INACTIVATION AND OF THE ADDITION OF COMPLEMENT

Antiserum condition	Test antigen	Condition	Curve area	Per cent.
I60(1+0) native	*Cancer borealis* 3b	native	289	100
" "	" "	inactive	164	57
I60(4+1 saline) native	*Cancer borealis* 1b	native	234	100
" "	" "	inactive	151	65
" "	*Cancer borealis* 2b	native	249	100
" "	" "	inactive	120	48
" "	*Cancer borealis* 3b	native	179	100
" "	" "	inactive	102	57
" inactive	" "	inactive	25	14
I60(4+0) inactive then add 1 part of complement	" "	inactive	67	37

the *amounts* of antigen to be used in the tests. As long as the curves are complete, the antigens are present in comparable amounts. But the photron'er has not as yet solved the problem of the *quality* of the antigens tested. The fact is that different samples of the same species of antigens may show different amounts of reactivity with the same antiserum. This is true even with pooled sera. We have already reported that mammalian sera show a slow decrease in reactivity over a period of years even though kept in the icebox. Recently we have discovered that complement may be concerned in precipitin reactions contrary to the current understanding among immunologists. The results of tests involving the inactivation of the reagents and the addition of complement are shown in Table II.

We note from Table II that inactivation of the antigen for a half hour at 56° C. cuts down the turbidity to an average of 57 per cent. of its original value and that when both antigen and antiserum are inactivated the value was lowered to 14 per cent. Addition of complement, presumably in excess, brought about only a partial restoration of the reactivity. The general understanding among immunologists that inactivation has no effect on precipitin reactions is probably due to the crudity of techniques previously employed and should be corrected.

Other variables involved in the measurements given include the degree of specificity of the antisera. It was discovered long ago and has been abundantly confirmed by Wolfe (1939 and earlier) that prolonged immunization leads to the production of less discriminating antisera of broad range, whereas a short injection procedure involving small amounts of antigen leads to more discriminating antisera of narrower range. Obviously antisera of the same grade of specificity must be selected if comparable results are to be gained. The antisera used in this report were not standardized as to their specificity though they were produced in a comparable manner. In the future we hope to use standard antisera, selected with regard to their grade of specificity as demonstrated by their reaction with some particular homologous and heterologous antigens.

It is known also that lipoids may be concerned in precipitin reactions. Thus it was reported (Boyden, 1936) that convergent ring test results were obtained in Crustacea, results which could be corrected by the removal of ether-soluble substances from the antigens. On the other hand, the photron'er comparisons with native sera appear to show little if any disturbance of this sort.

When these and other sources of error in the photron'er method are corrected we should be able to use serologically equivalent antigens and antisera. Even at present we can see the trend of the results and gain our first approximations to the quantitative measurement of the relationships of animals as indicated by their sera.

There appears to be only one other recent study of Crustacean relationships involving precipitin reactions. A report by Clark and Burnet (1942) contains some interesting and valuable data regarding precipitin tests on Australian Crustacea. Using the ring test or a crude flocculation test involving no measurements they find a general parallelism between the amounts of precipitate formed and the systematic positions of the species tested. They call attention to the relatively narrow range of their antisera, apparently not realizing that the inactivation of all their antigens and antisera must have considerably cut down the reactivity of their reagents. Absorption experiments were used to differentiate closely related species. Tests with the purified haemocyanin of one species disclosed the haemocyanin as the principal if not the only antigen contained in that serum. This result accords with the analyses of Allison and Cole on other species of Crustacea. The data thus indicate that with a more quantitative technique consistent measurements of the serum relationships of these species could be obtained. From the standpoint of technique the few tests on Crustacean sera performed by von Dungern (1903), Nuttall and Graham-Smith (1904) and Erhardt (1929) need not be examined at length.

SEROLOGICAL MEASUREMENTS IN RELATION TO SYSTEMATIC CATEGORIES

To date the photron'er has been, or is being, applied to the study of Mollusca (Chestnut, unpub.) Crustacea, Insecta (Leone, unpub.) Pisces (Gemeroy, in press), Aves (De Falco) and Mammalia (Boyden, 1942 and unpub.). Essentially the same types of results have been obtained in all these groups, and no one by looking at a series of curves could tell from which group of animals the curves were derived. More than that the amounts of serological divergence run, in general, in accordance with the rank of the systematic category, even though in not a single one of these studies was any attempt made to select antisera of certain grades of specificity. Thus to take data from

the comparison of common Mammalia we find the following values for their serological resemblances:

I. For members of the same genus
   Horse *vs.* ass, 85 per cent. (Boyden, unpub.).
II. For related genera of the same family:
   Brook trout *vs.* Rainbow trout, 66 per cent. ⎫
   Brook trout *vs.* Brown trout, 53 per cent.   ⎬ (Gemeroy, in press)
   Black bass *vs.* Large mouth bass, 75 per cent. ⎭
   Bison *vs.* cow, 73 per cent. (Boyden and De Falco, in press)
III. For closely related families:
   Bovidae *vs.* Cervidae, 45 per cent. (Boyden, unpub.)
IV. For distantly related families:
   Bovidae *vs.* Suidae, 5 per cent.

In Aves (De Falco, 1942) the values are generally higher in all the distantly related species, for ordinary antisera can apparently react with all the orders of birds suggesting that the amount of serological differentiation between orders of birds is roughly comparable to that between the more distantly related families of mammals.

The conclusion from this brief consideration of "serological dimensions" appears to be justified that precipitating antisera produced in rabbits may be used as a kind of taxonomic "yardstick" for measuring the magnitude and differentiation of systematic categories. It may even be possible with standardized antisera to set up limits for defining systematic categories and thus aid in the eternal debate between the "splitters" and the "lumpers." At any rate, the photron'er has thus far given some rather promising data, indicating that it may be useful in the arrangement of species within a genus, of related genera and of related families. In birds it may also aid in the difficult taxonomic problem of arranging the orders in accordance with a natural system.

The fact that the serological measurements fall off with the more distant systematic categories in a fairly regular way may also have a bearing on the nature of the evolutionary processes which have produced the various types of organisms in these categories. It has been ably maintained by Mayr (1942) that the evolution of subspecific and superspecific types is essentially the same type of process. On the contrary, Goldschmidt (1940) claims

that there is a difference in kind and in mechanism between these two grades of evolutionary divergence. So far as the serological tests discussed in this report go, they appear to confirm Mayr, for they indicate an apparently continuous evolutionary differentiation of the serum proteins. Thus individual serological variation grades into specific and specific into generic differentiation, with the amount of difference a matter of degree rather than of kind for all the systematic categories.

These general conclusions are supported by the recent valuable contributions of Cumley (1940), of Wilhelmi (1940, 1942) and of Wolfe (1939b). A recent review (Boyden, 1942) discusses these and other results in some detail, presents a critique of the principles of systematic zoology and discusses the relation of systematics to general biology from the point of view of a serological attack on these problems.

It is a marvelous thing that any animal, even such as the rabbit, should possess the capacity to produce, under appropriate conditions, antisera of innumerable kinds, each capable of reacting with a variety of chemically related substances in accordance with their chemical and serological resemblances. So far, the rabbit has actually made fewer mistakes than man in the attempt to construct a natural system of classification, for the serological data derived from the recent and more effective use of rabbit antisera have given a more consistent basis for the determination of animal relationship than has previously been developed on other grounds by men whose views regarding some of the more distant systematic relationships have been and still are widely divergent.

SUMMARY

The photron'er method has now been applied to the study of systematic relationships in representative Crustacea, Insecta, Pisces, Aves and Mammalia. This method of serological comparison has definite advantages over previous methods of study; in particular, it has solved the problem of the serological equivalence of antigens as far

as the *amount* of such antigens is concerned. The data reported as a result of these studies indicate that the photron'er technique may be usefully applied to the comparison of the species of a genus, the genera of a family and to the families of an order. In birds the method may also be used in the comparison of representatives of related orders. The·data from these various studies have led to a first quantitative approximation of the amounts of serological divergence characteristic of systematic categories of different rank and indicate that such data may provide a kind of serological "yardstick" for the delimitation of systematic categories. Further improvements in technique and a wider application of the method are needed if we are to make rapid progress in the application of serological techniques to animal systematics.

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QUANTITATIVE GENIC–HORMONE INTER-ACTIONS IN THE FOWL

I. RELATIVE SENSITIVITY OF FIVE BREEDS TO AN ANTERIOR PITUITARY EXTRACT POSSESSING BOTH THYROTROPIC AND GONADOTROPIC PROPERTIES

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IN previous publications from this laboratory (Munro, 1936; Munro, Bird and Hopkins, 1937) it has been suggested not only that the number of eggs produced by domestic fowl during their first laying year is very largely determined by non-genetic factors but also that that portion of the variance caused by genetic factors (20–25 per cent.) is the result of the action of genes which could be divided roughly into two sorts. First, the "primary" egg genes which are concerned with fundamental physiological processes, e.g., functional level of endocrine glands, sensitivity of soma to sex endocrines, efficiency of feed utilization, etc., and second, the "secondary" or "protective" genes which enable the organism to ward off disease or to function normally or more normally than individuals not possessing them, under such adverse conditions as deficient rations, extremes of temperature, etc. Since the environmental variations with which the protective genes interact are changing continuously, then egg production, in so far as it is influenced by these protective genes, will fluctuate in accordance with the interaction between the individual's complement of protective genes and the changing environment. On the other hand, it would seem that the primary egg genes should function more or less continuously under varying conditions but their actions, as measured by rate of egg production, would be, in most cases, almost completely masked by both purely environmental factors and the action of the protective genes.

It would appear that evidence for the existence of such primary genes might better be obtained by the use of other criteria than the egg production rate of adult females. For instance, if a breed or strain possesses a genetically determined low threshold of response to gonadotropic hormone, then administration of the hormone to responsive individuals of that breed should result in a greater physiological effect than similar administrations to a breed possessing a higher response threshold. If such breed differences could be proved, one would be justified in inferring that gonad development in some breeds require less of the native gonadotropin than others from which it would appear to follow that in these breeds egg production would be initiated at lower levels of pituitary activity.

There is considerable evidence showing that qualitative breed and varietal differences in feather morphology and coloration are controlled by intracellular (genic) rather than extracellular (humoral) factors (see review by Danforth, 1939). On the other hand, evidence on the relative influence of genic and humoral factors on quantitative physiological reactions is scanty. However, there is a growing list of evidence that such characters are also under genic rather than humoral control. Among these may be mentioned the differences between the comb of the Leghorn and the Plymouth Rock in degree of response to androsterone (Callow and Parkes, 1935); the findings of Bates, Riddle and Lahr (1939) with respect to differences between breeds of pigeons in the degree of crop-sac response to prolactin; the fact that animals from different colonies may vary widely in their sensitivity to gonadotropin (Barlow and Sprague, 1941). The report by Byerly and Burrows (1936) indicating that the pituitaries of broody breeds of chickens contain more prolactin than those of non-broody genetic constitution is one of the few indicating that functional physiological traits of a genetic nature are humorally controlled. In order to gain evidence for the existence of the postulated

primary egg genes as well as to add to existing knowledge concerning the control of physiological traits a series of studies was undertaken at this laboratory to determine the effect of the genotype on hormone reaction in the fowl. The present paper deals with breed differences in the response of certain primary and secondary sex organs to the injection of a pituitary extract containing both thyrotropic and gonadotropic fractions.

MATERIALS AND METHODS

Baby chicks were chosen in preference to older birds for a number of reasons: (1) They are easier and less expensive to produce in quantity; (2) they require less hormone, and (3) they should make better experimental material since the action of any extraneous hormones used would be interfered with little by endogenous hormones from their comparatively inactive sex organs. Five popular breeds, White Leghorn, New Hampshire, Light Sussex, White Plymouth Rock and White Wyandottes, were chosen. These are all single comb with the exception of White Wyandotte, which is a rose-comb breed. White Rocks were included because of the report of Nalbandov and Casida (1940) who found the immature testis of this breed to exceed the White Leghorn in sensitivity.

It is obviously impossible to obtain a representative cross-section of any one breed and still keep the number of individuals involved within the physical limits of an experiment of this sort. The chicks were obtained from one of the largest commercial hatcheries in Canada and they are therefore about as representative of their breed as it is possible to get without greatly increasing the numbers and adopting a method of restricted or stratified sampling. Upon arrival the chicks were placed in electrically heated brooders. The chicks were apportioned among five compartments in such a way, that each compartment had a fairly even representation of each breed. This was done to equalize environmental conditions

among the breeds. A six-day period of observation was allowed to elapse before treatment began, during which time all unthrifty individuals were removed. A total of 533 healthy chicks remained and were used in this study.

Injections were started on the seventh day, continuing daily for ten days. Prior to the commencement of the treatment period, each breed-group was divided into an experimental and a control lot. Each of these lots consisted of males and females in approximately equal proportions. Breeds, sexes and treatments were re-allotted at random to the five compartments.

The pituitary preparation employed consisted of a pooled sample of two chemically extracted dry powder concentrates of glandular origin, A.P. 61 B and A.P. 81 B, which in previous work on chicks in this laboratory had been found to possess chiefly gonadotropic and thyrotropic properties, respectively. They were very kindly supplied to the senior author by Dr. A. S. Parkes, of the National Institute for Medical Research, London, England. The relative biological potency of these extracts were determined in Dr. Parkes' laboratory with rats or mice as the test animal. However, unpublished work in our laboratory on a variety of extracts from the same source has shown that this potency does not agree with that determined on chicks. The extracts used in the present study were among the more potent in so far as their effects on chicks are concerned. The treatment consisted of a daily injection of 1.83 mg of the pooled extract dissolved in 1 cc of a slightly acidified aqueous solution which was brought to the neutral point just before injection. The total amount of A.P. concentrate supplied to an individual chick during the ten-day injection period was slightly over 18 mg. Control chicks received the solvent only.

Twenty-four hours following the last injection, the chicks were weighed and then killed by gas. The combs, thyroids and gonads were removed in both sexes. In the females, the oviduct was also excised. Upon removal,

the organs were weighed on a chainomatic balance to the nearest milligram.

Because of the intimate relationship between body and organ weights (Kosin, 1940), the latter had to be adjusted to a uniform body weight. Although in an earlier paper (Munro and Kosin, 1940) organ weights were expressed as a per cent. of body size, in the present work a different, and what is believed to be a more critical basis for comparison was adopted.

Hopkins and Biely (1935) concluded that as the size of the bird increases, the average percentage of the total body weight due to liver, kidney or spleen decreased. Data collected in this laboratory show that this tendency for an organ weight to become relatively smaller (actually larger in absolute terms) as the body weight increases also holds true for most of the endocrine organs in young fowl. Thus, the larger birds would be automatically penalized in any experiment in which organ weights were being studied and more especially it would be impossible to make a valid comparison of organ weights between breeds differing in size.

The difficulty can be overcome by correcting the mean organ weights of the various groups to be compared to a common body weight by means of the regression equation which characterizes each group. This, in effect, provides a measure of organ weight which, in theory, would occur if all birds were equal in body weight. The variability between organ weights which still exists is a net variability freed from the influence of variation in body weight, and the corrected means for the various groups thus secured have correspondingly reduced standard errors. These reduced standard errors, technically known as standard errors of estimate, are used to calculate the significance of the differences between the corrected means in the usual way. This procedure can be found outlined in any modern statistical text and is lucidly explained by Wallace and Snedecor (1931). Table 1 illustrates the relative standing of the five breeds

used in this experiment when (a) the organ weights are corrected to a common body weight by the method of regression and (b) the organ weights are expressed as a per cent. of the body weight.

It can be seen that the relative order of sensitivity is not the same in the two methods. We consider the re-

TABLE 1

ORDER OF BREED SENSITIVITY TO A.P. EXTRACT ACCORDING TO TWO METHODS OF COMPARISON. (ROMAN NUMERALS = ORGAN WEIGHTS CORRECTED TO UNIFORM BODY WEIGHT BY REGRESSION. ARABIC FIGURES = ORGAN WEIGHTS EXPRESSED AS PERCENTAGE OF BODY WEIGHT)

Organ	Comb		Thyroid				Gonad	
Sex	♂ ♂		♂ ♂		♀ ♀		♂ ♂	
White Leghorn	I	1	III	5	III	2	III	4
White Wyandotte	IV	5	I	1	1	1	II	2
Light Sussex	V	4	II	2	IV	4	IV	3
New Hampshire	II	2	IV	4	II	3	V	5
White Rock	III	3	V	3	V	5	I	1

gression method to be the better one and it has been employed in analyzing the data presented in this paper.

RESULTS

One of the first things which emerged from this study is the fact that basic differences exist in the size of the same organs in the control birds of different breeds. At seventeen days of age, the White Leghorn chicks of both sexes had the heaviest combs, thyroids and gonads, while the females of this breed also had the heaviest oviducts (Table 2). Because of this fact it is debatable whether the measure of breed sensitivity should be the simple excess of injected over control organs or whether this excess should be expressed in terms of the control. For instance, the injected Leghorns showed an increase of 25 mg over the control in weight of comb, while the corresponding increase for New Hampshires was only 12 mg. However, the 12 mg increase shown by the New Hampshires represents an increase of 66.7 per cent. over the controls, whereas the 25 mg increase in the Leghorns is only 62.7 per cent. higher than the controls. The data are summarized in Table 2, and it will be seen that the

TABLE 2

A SUMMARY OF THE DATA SHOWING THE CORRECTED ORGAN WEIGHTS FOR EACH BREED AND SEX GROUP. IN EACH COLUMN THE BREEDS ARE ARRANGED IN DESCENDING ORDER OF ORGAN SIZE AND IN THE CASE OF INJECTED BIRDS THE ABSOLUTE AND PERCENT INCREASE OVER THE CONTROLS IS ALSO SHOWN

W.L.—White Leghorn; N.H.—New Hampshire; L.S.—Light Sussex; W.R.—White Rock; W.W.—White Wyandotte

Comb				Thyroid				Gonad				Oviduct	
Male		Female		Male		Female		Male		Female		Female	
Injected	Controls	Injected	Controls	Injected	Controls	Injected	Controls	Injected	Controls	Injected	Controls	Injected	Controls
WL 63±3.7 +25±5.16 +62.5%	WL 40±3.6	WL 33±1.4 +4 +13.8%	WL 29±1.6	WW 17.7±1.3 +7.0±1.36 +65.4%	WL 10.9±0.8	WW 19.3±1.3 +8.5±1.36 +78.7%	WL 12.1±0.7	WL 70±2.9 +32±3.18 +84.2%	WL 38±1.3	NH 53±2.8 +22±3.22 +71.0%	WL 34±2.1	WL 27±1.1 +2 +8.0%	WL 25±1.0
WW 39±1.2 +7±2.08 +21.9%	WW 32±1.7	WW 28±1.5 +2 +7.7%	WW 26±1.9	LS 14.2±0.6 +3.9±0.78 +37.9%	WW 10.7±0.4	LS 16.7±0.9 +4.6±0.98 +38.0%	LS 11.1±0.4	WL 70±5.2 +42±5.65 +150.0%	NH 36±2.6	WL 50±2.2 +16±3.04 +47.1%	NH 31±1.6	WW 23±0.9 +1 +4.6%	WR 23±1.7
LS 32±2.4 +8±2.78 +45.5%	LS 26±1.4	LS 22±1.3 -2 +10.0%	NH 21±1.6	WL 14.1±0.6 +3.2±1.0 +29.4%	LS 10.3±0.5	NH 16.2±1.1 +5.8±1.17 +55.8%	WW 10.8±0.4	WW 66±3.7 +35±3.99 +112.9%	WW 31±1.5	LS 43±3.9 +16±4.25 +59.3%	LS 27±1.7	WR 22±1.4 -1 -4.4%	WW 22±0.6
NH 30±2.6 +12±2.67 +66.7%	NH 18±0.6	NH 21±1.5 +0 +0.0%	LS 20±1.1	NH 11.8±0.4 +2.5±0.57 +26.9%	NH 9.3±0.4	LS 14.7±0.7 +3.6±0.81 +32.4%	WR 10.6±1.2	NH 60±3.8 +24±4.61 +66.7%	WR 28±2.2	WW 40±2.1 +16±2.90 +66.7%	WW 24±2.0	NH 20±1.3 +2 +11.1%	NH 18±0.9
WR 24±2.2 +8±2.34 +50.0%	WR 16±0.8	WR 14±0.8 +0 +0.0%	WR 14±0.6	WR 11.4±0.8 +2.5±1.0 +28.1%	WR 8.9±0.6	WR 12.1±0.6 +1.5±1.34 +14.2%	NH 10.4±0.4	LS 58±3.3 +30±3.71 +107.1%	LS 28±1.7	WR 38±3.4 +14±3.80 +58.3%	WR 24±1.7	LS 18±1.3 +1 +5.9%	LS 17±1.3

NOTE: The number of chicks contributing to the data in each cell of this table varies from 20 to 35 with an average of 27.

TABLE 3

ORDER OF RANK WITH RESPECT TO DEGREE OF RESPONSE

(a) Based on absolute increase over controls; (b) based on per cent. increase over controls

Rank	Comb				Thyroid				Gonad			
	♂ ♂		♀ ♀		♂ ♂		♀ ♀		♂ ♂		♀ ♀	
	a	b	a	b	a	b	a	b	a	b	a	b
1.	W.L.	N.H.	W.L.	W.L.	W.W.	W.W.	W.W.	W.W.	W.R.	W.R.	N.H.	N.H.
2.	N.H.	W.L.	L.S.*	L.S.	L.S.	L.S.	N.H.	N.H.	W.W.	W.W.	W.W.*	W.W.
3.	W.R.	W.R.	W.W.*	W.W.	W.L.	W.L.	W.L.	W.L.	W.L.	L.S.	L.S.*	L.S.
4.	W.W.	L.S.	N.H.	N.H.*	W.R.*	W.R.	L.S.	L.S.	L.S.	W.L.	W.L.*	W.R.
5.	L.S.	W.W.	W.R.	W.R.*	N.H.*	N.H.	W.R.	W.R.	N.H.	N.H.	W.R.	W.L.

* Equal in rank within the column.

corrected weight of the organ which is the top figure in each cell is followed, in the case of injected birds, with the simple excess over controls and this in turn is followed by the per cent. excess over controls.   In each column the breeds are arranged in descending order of organ size, which does not necessarily correspond with descending order of sensitivity.   Table 3 shows the order of rank of the various breeds with respect to sensitivity for each of the organs studied.   The breeds are ranked (a) on the basis of absolute increase over controls and (b) according to per cent. increase over controls.

In Table 4 both the absolute and percentage differences in response between the various breeds are listed for each organ and each sex together with their standard errors. Because of the fact that each of these differences is based on four independent populations, *viz.*, an injected and a control group in each of the two breeds being compared, the standard errors are in many cases comparatively large.   An asterisk means that the ''t'' value is between the .05 and .01 error points, while a dagger means that the odds on significance is greater than 99 : 1.

Fig. 1 shows the corrected organ weights of the control and injected birds for each breed and each organ in each sex.   It provides a bird's-eye view of the results and enables one to make a rapid comparison of the relative weights of the different organs in the different breeds for both injected and control birds.   Fig. 2 illustrates the relative degree of response in the different breeds.   It provides a picture of how the breeds compare in sensitivity in a clearer manner than does Fig. 1.   It shows the absolute increase in organ weight immediately followed by the per cent. increase, each being drawn to its respective scale.

Comb

As was expected, the large-combed sexually precocious Leghorn exceeded all others in its absolute comb increase. In three of the four cases (see Table 4) the odds on sig-

TABLE 4

DIFFERENCES IN THE DEGREE OF RESPONSE BETWEEN ALL POSSIBLE BREED PAIRS FOR EACH ORGAN EXCEPT OVIDUCT. THESE DIFFERENCES ARE SHOWN IN BOTH ABSOLUTE AND RELATIVE TERMS. THE BREEDS LISTED ON THE TOP EXCEED THOSE IN THE MARGIN BY THE AMOUNTS SHOWN IN EACH CASE

Absolute difference

	W.L.	W.W.	L.S.	N.H.	
W.W.	† 18 ± 5.6				**♂♂ Combs**
L.S.	† 19 ± 5.9	.1 ± 3.5			
N.H.	* 13 ± 5.8	− 5 ± 3.4	6 ± 3.9		
W.R.	† 17 ± 5.7	− 1 ± 3.1	2 ± 3.6	4 ± 3.6	
W.W.	* − 3.8 ± 1.7				**♂♂ Thyroids**
L.S.	− 0.7 ± 1.2	* 3.1 ± 1.6			
N.H.	− 0.7 ± 1.1	† 4.5 ± 1.5	1.4 ± 3.1		
W.R.	† 0.7 ± 1.0	† 4.5 ± 1.7	1.4 ± 1.3	0.0	
W.W.	* − 3.9 ± 1.7				**♀♀ Thyroids**
L.S.	1.0 ± 1.3	* 4.9 ± 1.8			
N.H.	− 1.2 ± 1.5	2.7 ± 1.8	− 2.2 ± 1.4		
W.R.	† 3.1 ± 1.7	† 7.0 ± 1.9	2.1 ± 1.6	* 4.3 ± 1.8	
W.W.	− 3 ± 5.1				**♂♂ Gonads**
L.S.	2 ± 4.9	.5 ± 5.5			
N.H.	8 ± 5.6	11 ± 6.1	6.0 ± 5.9		
W.R.	− 10 ± 6.5	7 ± 6.9	− 12 ± 6.8	† − 18 ± 7.3	
W.W.	0.0				**♀♀ Gonads**
L.S.	− 6.0 ± 4.4	0.0			
N.H.	2 ± 4.9	− 6.0 ± 4.3	− 6.0 ± 5.3		
W.R.		2 ± 4.8	2 ± 5.7	8 ± 5.0	

Percentage difference

	W.L.	W.W.	L.S.	N.H.
W.W.	* 40.6 ± 18.9			
L.S.	† 17.0 ± 21.9	− 23.6 ± 15.4		
N.H.	− 4.2 ± 23.2	† − 44.8 ± 17.2	− 21.2 ± 20.5	
W.R.	12.5 ± 23.3	− 21.8 ± 17.4	− 4.5 ± 20.6	16.7 ± 22.0
W.W.	* − 36.0 ± 17.5			
L.S.	8.5 ± 14.1	27.5 ± 16.3		
N.H.	2.5 ± 13.2	* 38.5 ± 15.5	11.0 ± 11.5	
W.R.	1.3 ± 16.6	* 37.3 ± 18.5	9.8 ± 15.3	− 1.2 ± 14.5
W.W.	† − 40.7 ± 17.5			
L.S.	5.6 ± 13.5	† 46.3 ± 16.3		
N.H.	− 17.8 ± 13.2	22.9 ± 18.3	− 23.4 ± 14.5	
W.R.	23.8 ± 17.9	† 64.5 ± 19.7	18.2 ± 16.2	* 41.6 ± 18.6
W.W.	− 28.7 ± 18.6			
L.S.	− 22.9 ± 19.9	5.8 ± 23.4		
N.H.	− 17.5 ± 18.8	* 46.2 ± 22.5	40.4 ± 23.5	
W.R.	* − 65.8 ± 28.8	− 37.1 ± 31.3	− 42.9 ± 32.1	† − 63.3 ± 31.4
W.W.	− 19.6 ± 19.2			
L.S.	− 12.2 ± 20.3	7.4 ± 24.1		
N.H.	− 23.9 ± 16.1	− 4.3 ± 20.1	− 11.7 ± 21.7	
W.R.	− 11.2 ± 20.7	8.4 ± 24.4	1.0 ± 25.2	12.7 ± 22.0

* Odds on significance > 19 : 1 ;   † Odds > 99 : 1

nificance exceed 99:1. However, so far as percentage increases are concerned the Leghorn excess reaches the point of statistical significance only when compared with the low-ranking White Wyandotte. The most surprising thing in connection with the comb is the very large percentage increase in the New Hampshires, which was 67 per cent. as compared to 63 for the Leghorns. The odds on significance of the difference between New Hampshire and White Wyandotte exceeds 99:1. The White Rocks and Light Sussex with gains of 8 mg (50 per cent.) and 6 mg (45 per cent.), respectively, followed the Leghorns and New Hampshires but exceeded the White Wyandotte which showed a gain of 7 mg or only 22 per cent.

By comparison the females showed little stimulation. The Leghorns showed a gain of 4 mg or 14 per cent.; the Light Sussex, 2 mg (10 per cent.); the Wyandottes, 2 mg (8 per cent.) while the New Hampshires and White Rocks were not affected by the injections. In connection with the combs it is interesting to note that, in both sexes, the Leghorn has the largest natural comb weight, followed by the Wyandotte, with the White Rock bringing up the rear. In males, the Light Sussex and New Hampshires take third and fourth place, respectively, while in the females these two breeds are about equal.

THYROID

None of the basic breed differences in the thyroid weights of the control groups of either sex were statistically significant, but the injected chicks showed distinct breed differences in thyroid response. The Wyandotte is by far the most sensitive. Both sexes of the breed surpassed the other four in both absolute and relative thyroid response and in all cases except that of New Hampshire females the differences are greater than required for odds of 19:1. Gains of 65 per cent. and 79 per cent. in the Wyandotte males and females, respectively, were recorded as compared to 38 per cent. for

Light Sussex males and 56 per cent. for New Hampshire females which were next in order of gain in their respective sex groups. The Light Sussex males, however, are not significantly higher in response than any of the remaining three breeds, but the New Hampshire females do exceed the significant point both absolutely and relatively when compared with the low-ranking White Rock females. White Leghorns, which are generally used in thyrotropic assays, were third on the thyroid sensitivity list with White Rocks at the bottom.

A cursory examination of the thyroid data indicated the existence of a sex difference in the size of that organ. This was confirmed on analysis. The pullet chicks of the control lots averaged 1 mg heavier in thyroid weight, when comparisons were made within breeds. The odds on significance tested by "Students" method is approximately 99 : 1. There is also a distinct though statistically non-significant tendency for the degree of thyroid response in female to exceed that in males.

GONADS

While White Rocks were among the least responsive to hormone injections in so far as the comb and thyroid tissues were concerned, the testes of this breed showed an inordinately large increase. Although normally possessing testes fully one third smaller than those of the Leghorns, the male gonads of the White Rock chicks showed a response of 150 per cent. This greatly exceeds the 84 per cent. response of the Leghorn testes. In fact, the White Leghorn rate of response was second lowest, surpassing only that of the New Hampshires. Table 4 shows that while the White Rock testes consistently exceeds all others in both absolute and relative response, this excess reaches the significant point only when compared with the New Hampshire (absolutely and relatively) and the White Leghorn (relatively). The White Wyandotte testes give the second highest response, *viz.*, 35 mg or 113 per cent. This response is significantly higher than the low-ranking New Hampshire.

The control groups of all breeds had smaller testes than the Leghorns. With the exception of the New Hampshire testes these differences were statistically significant. This same breed relationship is found in the ovaries.

Compared to the testes, response of the ovaries was, on the whole, a good deal lower. Here the New Hampshire pullet chicks showed the greatest degree of response when measured in both absolute and relative terms. The Leghorns, which normally possess the heaviest ovaries, failed to keep the lead under hormone stimulation. However, none of the breed differences were statistically significant.

OVIDUCTS

The oviducts were practically unaffected by the treatment, as can readily be seen by referring to Fig. 1. In only three of the five breeds do the treated birds exceed the controls. Apparently the ovaries were not stimulated to elaborate estrogen at least in effective amounts.

EFFECT OF FAST-FEATHERING GENE

Because of the appearance of fast-feathering individuals in the predominantly slow-feathering breeds, such as White Wyandottes and White Rocks, an opportunity presented itself to test for the existence of any relationship between this gene and the rate of response to hormone. The sex-linked gene for slow feathering (K) is dominant to the fast-feathering gene (k). Results of the analysis of the data showed that at least under the conditions of this experiment, these two alleles do not differentially affect the response threshold in any of the organs studied.

DISCUSSION

Two parallel series of results emerge from this study. First, there exist basic breed differences in the size of certain primary and secondary sex organs of baby chicks. Breneman (1941) found such differences in the organs of

White Leghorns and Rhode Island Reds. He suggests that such differences be taken into consideration when hormone experiments are based on chicks belonging to several breeds.

The second point concerns itself with what are apparently genetically controlled differences in the response of these organs to the A.P. extracts. This breed specificity in response often overshadows the normal standing of breeds in respect to the natural size of these organs. Breeds which possess naturally heavier organs do not necessarily exhibit correspondingly higher or lower degrees of response.

Perhaps the most striking case in this connection is that of White Wyandottes. The thyroids of this rose-combed breed apparently have the lowest response threshold of any of the five breeds studied. The fact that both sexes of this breed showed the highest sensitivity lends strong support to the thesis that this is due to a gene control over the somatic threshold of response to thyrotropic hormone.

The White Rock breed presents another example of this phenomenon. Largely because of the naturally small size of comb, thyroid and ovary, the weights of these organs in the hormone-treated groups of this breed were, relatively, the smallest of the five breeds tested. Moreover, the rate of response was also of a uniformly low order. The White Rock testis, on the other hand, although also the smallest of the control groups, showed an extremely high sensitivity with an increase of 42 mg or 150 per cent. These are considerably higher than corresponding values for the Leghorns and confirms the findings of Nalbandov and Casida (1940) in respect to the relative sensitivity of these two breeds.

The high level of response exhibited by the White Rock testis is all the more surprising in view of past experiences in this laboratory with a closely related variety, the Barred Rock. The latter proved to be less suitable than Leghorns for work on the gonadotropins because of

its high response threshold. It is difficult to account for such a distinct intra-breed response differential, particularly since the White Rock is known to have originated as a recessive white mutant from the Barred variety. Originally, then, the two varieties differed in only one gene, the White Rock lacking the chromogen-forming dominant gene C possessed by the Barred. More recently certain strains of White Rocks have been altered by introducing White Wyandotte and White Leghorn blood with the result that some families and strains are known to lack the barring gene while some possess dominant white plumage; they also, undoubtedly, differ with respect to other cryptomeres. All this complicates the picture, but it is still possible that the lower threshold value of the White Rock testis is due to the original single gene difference between the Barred and White varieties. The relatively high sensitivity of the White Wyandotte testis, another breed which lacks the C gene, lends weight to this possibility. Plans are under way in this laboratory to test the relationship between this gene and testis sensitivity.

The low threshold of response of the White Rock testes contrasts sharply with the relatively low degree of sensitivity of the male chick combs in this breed. At the same time, combs of the male Leghorn chicks responded at a high rate compared to their testis tissue. This may indicate the existence of purely local organ specific response thresholds, or it is possible that a mere increase in testis size is no criterion of the amount of endogenous androgen which it produces. It also seems likely that the genotype exercises a control over ultimate organ and body size. Thus, no amount of androgen will make the Barred Rock male comb as large as that of a normal White Leghorn male.

The existence of a sex difference in the weight of the thyroid gland presented some interest in light of the earlier report of Riddle (1929), who found no significant sex difference in the weight of this organ in pigeons and

doves. Juhn and Mitchell (1929) presented inconclusive evidence on this point in adult Brown Leghorns. Findings of the present investigation are in agreement with those of Aberle and Landauer (1935), who showed that female chicks of the White Leghorn and Frizzle breeds have larger thyroids than the males.

The results of this investigation suggest some practical considerations. The fact that there exists inherent breed specific degrees of organ response to hypophyseal hormones should cause investigators to exercise greater care in the selection of breeds and varieties of chickens for use in endocrine investigations. Additional data being accumulated at this laboratory indicate that the same principle of a genetically controlled response in chicks exists with regard to both estrogens and androgens. The judicious selection of breeds should greatly increase the efficiency of hormones when chicks are used as experimental animals. It is entirely possible of course that other breeds or varieties exist which will prove more efficient in this type of work than those found most responsive in the present study.

Another point which may prove of practical value is the possibility that breeds, and more especially families within breeds, which are most sensitive to gonadotropins may prove to be superior in reproductive performance. For example, the New Hampshire females in this study have the most responsive ovaries, and although the differences between this breed and the others do not reach the point of statistical significance, there is good evidence that the New Hampshire is superior in its ovarian response. Might not this breed, in respect to its primary egg genes (referred to in the introduction), be genetically superior to the others? The laying ability of the New Hampshire has many champions, and although there is no unanimity with respect to this it does appear that the eggs of this breed hatch better than most others. Any superiority possessed by a breed by virtue of its complement of primary egg genes would be, as explained previ-

ously, largely masked by the protective genes and the environment. It would be difficult, therefore, to prove any relationship between, for instance, ovarian response to hormones and reproductive performance. However, this is a point which should be borne in mind.

Summary

Baby chicks of both sexes of five commercially popular breeds of fowl, *viz.*, White Leghorn, New Hampshire, Light Sussex, White Plymouth Rock and White Wyandotte, were compared in respect to the degree of response (increase in weight) shown by their gonads, thyroids and comb when injected with a total of 18 mg of concentrated anterior pituitary extract per chick spread over a ten-day injection period. Controls were run in each sex and breed group. Organ weights were corrected to a uniform body weight by means of regression.

Comparisons between the control groups showed that breed differences exist in the natural organ weights. After treatment the relative organ weights in the different breeds are often reversed due to a breed differential in sensitivity of response.

When the breeds are compared on the basis of either absolute or relative increase over their untreated controls, certain organs of certain breeds were outstanding in the degree of their response. Chief among these were: (1) the combs of White Leghorn males; (2) the thyroids of both sexes in Wyandottes; (3) the testes of White Wyandotte and especially White Rock males; (4) the ovaries of New Hampshire females, although this point can not be considered as fully established in this study.

Different strains of these breeds of course will not necessarily show the same relative response. However, this study shows that breeds differ in their response to pituitary hormones. These breed differences are considered to be due to a genic control over the somatic response threshold.

Some practical implications of these findings are discussed.

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REVIEWS AND COMMENTS

IN this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution.

REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

Family Treasures. A Study of the Inheritance of Normal Characteristics in Man. By DAVID L. WHITNEY. Lancaster, Pa.: The Jaques Cattell Press, "1942" [1943]: 1–299, figs. 1–234. $3.50.

THIS finely illustrated and interestingly written book was prepared "for amateurs in the study of human heredity and for those who are interested in personal inheritance of family traits. It is a simple presentation mainly by photographs of many of the normal traits appearing in families of two or more successive generations." It will therefore have a strong popular appeal, for the mind of man, when unfettered by scientific training, learns and generalizes from the sort of impressive examples that Whitney describes and figures. The "amateur" will seldom stop to query whether chance might explain familial resemblances in selected samples. He will be relieved at the almost total omission of pedigrees, correlations and ratios (the nearest approach to a statistical treatment lies in enumerating the results of the trial flippings of a coin and of the blind selection of pairs of marbles from a supply of black and white ones). Some critical folks may wonder whether such teaching by the precept of example may not diffuse a false conception of the scientific method. Disapproval will also come from an almost opposite quarter, from those social scientists who deny that heredity is significantly involved in determining the behavior, the temperament and the mental

abilities of the human individual. Without concealing his own naturalistic philosophy, Whitney compromises with the humanists in conservatively stating that human inheritance *seems* to follow the laws that operate in animals, and that man's ancestors were *probably* animals. He deals rather effectively and very amusingly with the common superstitions and myths concerning the role of providence and of the devil in human heredity, and with prenatal influences and the inheritance of acquired characters.

Levels of Integration in Biological and Social Systems. Edited by ROBERT REDFIELD. Biological Symposia, Vol. VIII. Lancaster, Pa.: The Jaques Cattell Press, 1942: i–v, 1–240, 7 figs. $2.50.

IN this significant contribution to the philosophy of biology and of sociology, ten scientists have presented their data and views on the social system and its biological precursors and analogs. An attempt to integrate their views is made by ROBERT REDFIELD.

The common thread in the contributions is the development on different biological levels of similar systems of integration, under which individuals become coordinated in larger units. The expanded units, it is repeatedly affirmed, possess attributes of the individual organism. The organismic concept is therefore stressed, particularly in Redfield's Introduction and in ALFRED E. EMERSON's chapter, Basic Comparisons of Human and Insect Societies. Essential features of the individual—life, origin, growth, survival in the struggle for existence, maturity, reproduction, and death—are indicated either by definite claims or by connotation to be characteristic also of the species and of the major phyletic lines; of integrated colonies; of ecological communities, and of various types of social groups. Parallels, as of mutual interdependence and division of labor, feature the integrated colony, including, as LIBBIE HYMAN reports, the metazoan individual; populations of lower pre-social animals, including

the infra-social insects, which are discussed from an ecological viewpoint by THOMAS PARK; the highly complex, heredity-based systems of insects; the social organization, dominated by rank-order, in birds and mammals, interestingly treated by W. C. ALLEE; the sub-human Societies of Monkeys and Apes—the subject of C. R. CARPENTER's contribution; the Societies of Primitive Man, presented by A. L. KROEBER; and Modern Society, by ROBERT E. PARK. Evolutionary advance in the successive categories of social systems is mentioned, even in an age when a cynic might be filled with doubts.

Distribution and Variation of the Horned Larks (Otocoris alpestris) of Western North America. By WILLIAM H. BEHLE. Univ. Calif. Publ. Zool., 46, 1942: 205–316, figs. 1–3. $1.25.

THE horned larks are shown to be highly variable. Their variation and differentiation is to a large degree individual, developmental and sexual, but is in part correlated with geography. Numerous geographical subspecies are therefore separable. These local forms occupy distinct faunal areas, life zones and physiographic areas. Subspeciation is tied in with environmental changes, as of climate, and is regarded as "a continual process of adjustment and adaptation in the organism, which has resulted in continued harmony, or approach to harmony, between the organism and its environment." Racial colors tend to correspond adaptively with soil colors, as they do in African larks. The breakdown of subspecies into minor local races and the close agreement between some geographically remote subspecies is perhaps to be explained on the basis of such adaptations in color. In other ways the local forms show adaptations to their particular habitats, and the horned larks as a group are strikingly adapted for life on open areas with a small amount of vegetational cover. Of the "ecological laws," Bergman's and Gloger's apply, but Allen's does not.

Behle breaks away in a restrained fashion from or-
nithological precedent. His treatment is more detailed
than usual but is still in large part impressionistic. Cor-
relations between plumage color and soil color are af-
firmed but not measured. Critical speciational relations,
as in areas of subspecific intergradation, are extensively
discussed, without the test that quantitative data would
supply. Important advances are made toward the better
understanding of speciation, but with statistical support
these contributions would have been more securely estab-
lished and more convincingly presented.

**The Genus Nysius and Its Allies in the Hawaiian Islands
(Hemiptera, Lygaeidae, Orsillini). By ROBERT LESLIE
USINGER. Bishop Museum Bull. 173, 1942: i, 1–167, 9 text
figs., 12 pls. $2.00.**

IN the reviewer's opinion, this is the finest piece of
systematic work ever published concerning any group of
Polynesian insects. How much easier would be the tasks
of future workers if more authors would adopt a style
similar to Usinger's!

The bug-tribe Orsillini is spread throughout the world,
but its greatest proliferation is in Hawaii where there are
84 endemic forms contained in 5 genera (4 endemic). The
closest approach to the Hawaiian fauna in comparative
development and complexity is that of New Zealand (but
the two faunas are not directly related).

"The peculiar Hawaiian orsilline fauna exceeds all the
others in complexity and degree of divergence and may
reasonably be considered the oldest." The reviewer does
not believe that in insular faunas divergence and com-
plexity necessarily indicate great age as compared to
continental areas. Explosive speciation and great diver-
sification are characteristics of islands known to be
geologically young. The Hawaiian Orsillini have been
derived from ancestral immigrants, therefore they must
be younger than some other faunas.

"The endemic genera *Nescis, Oceanides* and *Glypto-nysius* probably migrated down the long series of leeward islands before the main islands of the present day were built." This is good reasoning, and it finds support in other groups of organisms. "They must have arrived not later than earliest Tertiary times as judged by mainland evolutionary rates, or perhaps later than this considering that evolution has taken place in the absence of severe competition." If Usinger means that the ancestors of these forms arrived in the main Hawaiian islands at such an early date, I believe that he is overestimating the ages of the islands. His second conclusion appears by far to be the most likely; his first is not supported by geological facts.

Usinger's conclusions are Neo-Darwinian. He finds gradations from widespread, variable species to scarcely differentiated forms to polytypic species to supra-species to geographical subgenera. One current school might say that Usinger is dealing with micro-evolutionary segregates and not "good species." However, a review of the text and a glance at the superb illustrations (which convey only a small part of the numerous differences of the living animals) should eliminate such thought. "The conclusion seems inevitable that geographical isolation or host isolation or both may be sufficient to set in operation the processes of species formation, while the biotic environment plays an all-important role in determining the rate and limits of this evolution. A disharmonic insular area with great gaps in its environment allows many non-lethal mutations to persist, whereas a fiercely competitive mainland environment rigidly rejects all but the best adapted, thus favoring adaptive evolution by natural selection." Such conclusions can hardly be escaped by open-minded students of mid-Pacific biotas.

Usinger wisely discards the worn-out opinion of those who believe the Hawaiian islands to be remnants of a drowned Pacific continent and logically adopts the "stepping stone" hypothesis and considers that migration has

taken place by short jumps from island to island along island chains. He also logically includes the decadent leeward islands as being of great importance as a migration lane which, by a circuitous route, leads to the Australian, Papuan and Oriental regions. This accounts "for the complete absence of many groups, such as the Orsillini, from southeastern Polynesia, the very islands where they would be expected to occur had the fauna of Hawaii been derived directly from the southwest." The relationships of the group show a derivation from the west and southwest Pacific. "An interesting anomaly is the lack of relationship with Micronesia and American species." Wind is considered to have been the principal agent in the dispersal of these small bugs.

Usinger believes that "To the evolutionist they represent the first case of tremendous proliferation of species in insular areas which presents a possibility of experimental analysis." The reviewer agrees, because a sound, adequate foundation is available; the habits, hosts and life histories of several species are known; there is every category from local varieties to genera to work with; and at least some of them are easily raised in captivity. We hope that the author may some day have an opportunity to carry on further research on this problem in Hawaii where it may lead to some most significant evolutionary facts.

ELWOOD C. ZIMMERMAN

NOTICES OF NEW BOOKS

Papers from Tortugas Laboratory, Volume XXXIII. Carn. Inst. Wash. Publ. 524, 1942: i–iii, 1–195, pls. 1–7, 73 figs. $1.50 (paper), $2.00 (cloth).—It is gratifying to see that the Papers from Tortugas Laboratory are still appearing. The thirty-third volume is made up of six contributions. HAROLD W. MANTER describes gasterostome trematodes from Tortugas. LEONARD B. CLARK and WALTER N. HESS describe in detail the swarming of the Atlantic palolo worm, concluding that "although reproduction occurs over a much longer period than was previously held,

it is thought that the lunar cycle, maturity of the palolo worm, and wave action are the three main factors determining the time of a major swarm." The same authors treat the responses of this worm to light, and correlate its light reactions with the uniform time of night at which it swarms. RALPH WICHTERMAN reports on the structure and division of three new ciliates from the littoral earthworm of Tortugas, and describes another new ciliate, representing a new family, and its symbiotic zooxanthellae. JOHN H. DAVIS, JR., concludes the volume with a long and interesting account of the vegetation and topography of the Sand Keys of Florida.

The Embryology of Eleutherodactylus nubicola, an Anuran Which Has No Tadpole Stage. By W. GARDNER LYNN. Carn. Inst. Wash. Publ. 541, 1942: 27–62, pls. 1–5, figs. 1–40.—This Jamaican leptodactylid deposits large unpigmented eggs on land. After a developmental period of about 26 days they hatch as completely formed frogs. The numerous developmental modifications include absence of external and internal gills, open gill slits, specialized larval mouthparts, coiled intestine, larval adhesive organs, and true operculum. Development of the chondrocranium and jaws is characterized by lack of the larval specializations normally occurring in leptodactylid tadpoles. Supra- and infrarostral cartilages are not differentiated, Meckel's cartilage is elongate, and the form and articular relations of the quadrate are essentially as in the adult. The larval quadratocranial commissure and processus muscularis do not appear. Lynn points out the scattered occurrence of direct development in the Salientia, but suggests that the factors bringing it about may be the same in each case. "It is suggested that many of the features which characterize the abbreviated 'larval' history may be brought about as a result of precocious activity of the thyroid gland, and further investigation of this aspect of the matter is now being carried out."—GRACE L. ORTON.

Picture Book of Insects. By ALBRO T. GAUL. New York: Lothrop, Lee & Shepard Co., 1943: 1–40, illustr. $1.50.—Designed for eight to twelve year olds, this book comprises a series of attractive plates, accompanied by text statements that remind one of the well-written labels of a good museum exhibit. It is well designed to catch the eye and to inspire interest in insects.

Meet the Natives. An Easy Way to Recognize Rocky Mountain Wildflowers, Trees and Shrubs of the Central Rocky Mountain Region. By M. WALTER PESMAN. Denver, Colo. (372 S. Humboldt St.) : Author's Edition, 1942: 1–216, many figs. $1.25. —"Meet the Natives" is, as the title implies, a pleasant and informal introduction to the wildflowers, trees and shrubs of the central Rocky Mountains. Instead of the usual type of keys employed in manuals, the plants are grouped first according to life zone, then arranged according to color of the flowers. To emphasize the color and make the book easier to use, colored paper corresponding to the flower color is used. The plants in the various sections are arranged according to flowering time, starting with the early blooming plants. Common and scientific names are both given and are followed by a brief characterization of the species. Water plants, weeds and vines are listed separately to facilitate identification. Many excellent photographs and line drawings are included. The drawings in particular emphasize the diagnostic features of the plants. For those who are interested primarily in the names of plants and the places they grow, this is an excellent introduction to the study. Young people whose interest in the out-of-doors is just being awakened will find in this book a charming and not at all formidable way to learn the plants. It should be a welcome addition to every camp and school library. The definitions of some frequently used scientific terms and the bibliography will be useful to those who are stimulated to further study. The small size and attractive appearance will appeal to the hiker and nature lover. The professional botanist will find it of interest primarily because of the photographs and drawings.—Mrs. HELEN V. SMITH.

SHORTER ARTICLES AND DISCUSSION

EVIDENCES OF CANNIBALISM IN THE TADPOLES OF THE FROG RANA PIPIENS

LARVAL salamanders frequently snap off each other's gills, legs or tails when hungry and crowded. According to Noble (1931) some terrestrial salamanders will leave part of their tail in the hand which attempts to seize them, a protective process somewhat similar to the autotomy of the claw of the lobster. The normal diet of frogs and toads may be animal or plant food, or both, depending partly upon the species and the habitat. Generally the larvae are vegetarian until metamorphosis, when the bulk of the food changes to Insects, Annelids and Arthropods. Many of the larger frogs (*i.e.*, bullfrogs) are cannibalistic in that they will eat smaller frogs such as the green or the leopard frogs. The tadpoles of *Ceratophrys ornata* are largely cannibalistic (Noble, 1931) but generally feed on the larvae of other frogs. Among the tadpoles there seems to be a definite food preference; in some it is exclusively vegetarian. Since all amphibia are voracious eaters whether as larvae or as adults, a sparcity of food accentuates the otherwise incidental cannibalistic tendencies of various forms.

The ability to regenerate is not related to the liability to injury. Newts may regenerate their hyoids (Bogoljubsky, 1924), frogs may regenerate their lungs (Westphal, 1925), and the exposed gills of the axolotl may be readily regenerated although not always in the same form (Wurmbach, 1926). The power of regeneration is gradually reduced in phylogeny (Korschelt, 1927). Larval regeneration is more frequent and more perfect than that of the adult amphibian, due in part to the forming of blastemas of more or less undifferentiated tissue which is therefore labile. Ubisch (1923) found that regeneration was in general better the more posterior the site on the larva.

Cannibalism among *Rana pipiens* tadpoles has been observed only in cultures where there are dead tadpoles that are devoured, along with other materials on the bottom of the containers. This paper presents evidence of true cannibalism among living tadpoles of this species.

Rana pipiens tadpoles were kept in groups of 100 in tanks measuring 24″ x 12″ x 6″ under controlled conditions of light and

PLATE I
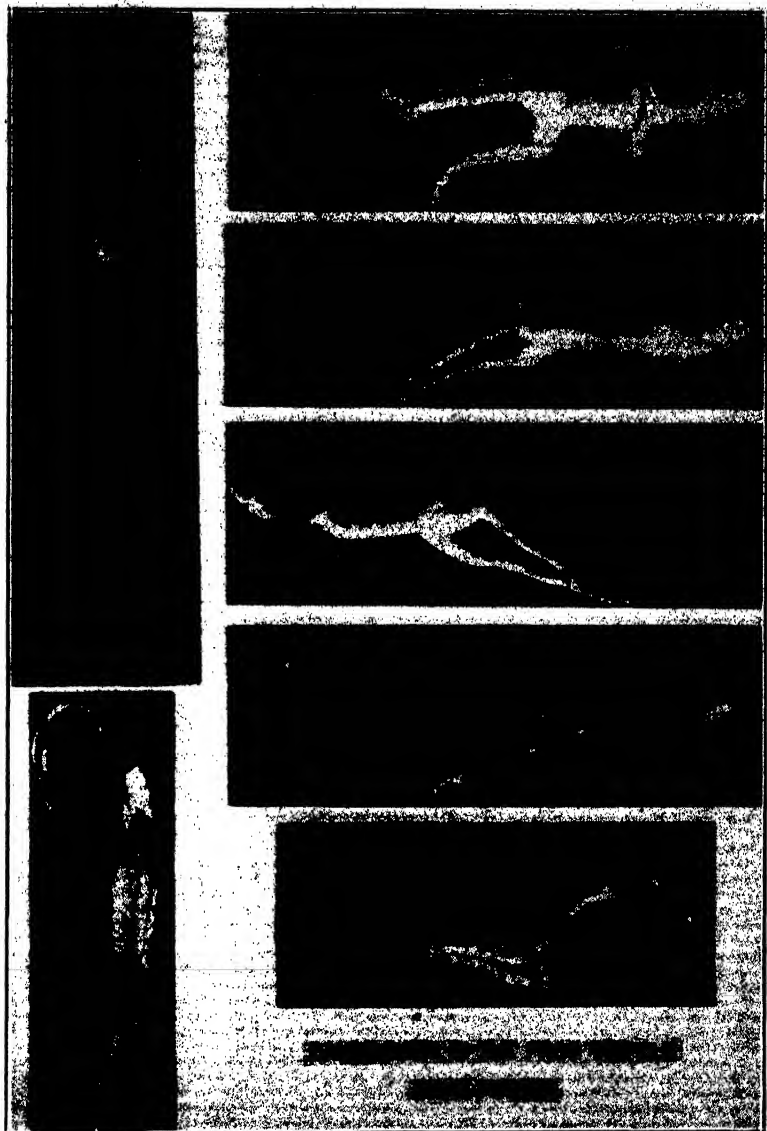
FIGS. A to D show various degrees of injury due to cannibalism in Rana pipiens. Regeneration of these areas is achieved by isolating the tadpoles in fresh water. FIGS. E to G show viscera protruding from the injured area, after cannibalistic attack. Such injuries rarely heal over, even under the best of conditions.

(Note: Same general position of all cannibalistic attacks.)

temperature. They were fed maximally on spinach which·had been thoroughly washed (to remove insecticides) and had been partially boiled (Rugh, 1941). The amount of cannibalism in the various tanks was not constant, but it occurred even under the most favorable conditions. The tadpoles were seen to nip each other quite readily, the chief sites being the head, the tail and the body wall just anterior to the junction of the tail. The head integument is relatively tough, while the tail is generally active, so that these two regions were the least vulnerable. The lesions were most frequently found postero-laterally on the body wall where the integument is rather thin and through which may be seen the highly colored liver and other viscera. They were almost invariably oval or rounded. The first appearance of the lesion is brown, due to the presence of underlying melanophores. Necrosis of the epidermis soon occurs, resulting in a white patch which is completely avascular. At this stage the melanophores and the xantholeucophores underlying the necrotic epidermis appear perfectly normal. The vascular pattern is quite normal except for a slight hyperaemia. Frequently the body wall is further perforated by continued nipping, resulting in death to the tadpole within six to twenty-four hours. When perforation of the muscular body wall is achieved and the viscera are visible, recovery is impossible.

The above conditions might well have escaped observation due to the rapid regenerative powers of the larval integument except for the fact that some of the tadpoles were subjected to an experimental environment in connection with a series of experiments on diet. To the spinach suspension was added $M/10^{-6}$ solution of methyl cyanide. This cyanide prevented proper regeneration of the integument, hence they accentuated the appearance of the lesions and made these observations possible. When such animals were returned to pure spring water and somewhat segregated, the lesions generally healed rather quickly. It has been conclusively demonstrated that the methyl cyanide in no way caused the lesions but once the integument was broken (by cannibalism or otherwise) this dilute cyanide solution prevented the normal regeneration of the tadpole integument.

The approximate size of the tadpole when cannibalism begins is about 65 mm in total length, 22 mm in body length, and 6 mm in hind-limb length. In an experimental group, poorly nourished on cabbage and treated with methyl cyanide, no lesions were seen.

This is explained on the assumption that cannibalism bears a relationship to the size of the tadpole and these starved tadpoles were relatively very small. Also, small larvae are able to move quickly and avert attack.

In experimental work with amphibian larvae it is of utmost importance that they be fed adequately and that they be not crowded, two conditions which, if not observed, will increase the probability of cannibalism.

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THE HISTOLOGICAL BASIS OF A SPECIFIC DIFFERENCE IN LEAF TEXTURE

IN a previous study of the species problem in *Uvularia* (Anderson and Whitaker, 1934), a working hypothesis was advanced to account for certain general differences between *U. perfoliata* and *U. grandiflora*. Since *Uvularia perfoliata* in flower, leaf and inflorescence is characteristically smaller and more graceful than *Uvularia grandiflora* (which by comparison seems coarse and irregular) it was suggested that this difference had its basis in a fundamental nuclear difference between the two species. Such a difference would be expressed, more or less similarly, in various parts of the plant. On this explanation the neater and more finished leaf texture of *U. perfoliata* would depend upon

the general tendency of that species to produce smaller, more regular cells.

When a few preliminary trials showed that the above hypothesis could be readily tested by the celloidin peel technique (Long and Clements, 1934), specimens of each species were selected from the herbarium of the Missouri Botanical Garden. Celloidin peels were made from the upper epidermis of a corresponding leaf from each plant. The outlines of the epidermal cells were then drawn with a camera lucida.

The results confirmed the hypothesis. The epidermal cells of *Uvularia grandiflora* tend to be larger, more irregular and more variable than those of *U. perfoliata*. The gross effect of this tendency to larger, more variable, more irregular cells is a tendency to a coarser, cruder leaf-texture.

SUMMARY

The differences in leaf texture between *U. grandiflora* and *U. perfoliata* depend on cellular differences. The larger, more irregular, more variable epidermal cells of *U. grandiflora* give it a coarser, cruder texture than that of *U. perfoliata*.

CONCLUSIONS

The results reported above provide concrete data on an aspect of the species problem which many naturalists have felt but few have discussed. Closely related species commonly differ from one another not only by a few trivial (albeit taxonomically useful) details but also by a host of vague tendencies variously expressed throughout the organism (Anderson and Whitaker, 1934; Anderson and Ownbey, 1939). This second sort of difference, however useless it may be to the taxonomist and however mystical it may appear to those without biological insight, rests upon a firm foundation theoretically. Species are now known to differ by their nuclei. Consequently in considering any two species one sees the same set of differences more or less harmoniously expressed throughout the organism. The same intracellular influences which produce a tendency towards a certain type of leaf produce correlated changes in the inflorescence. Though difficult to apprehend and even more difficult to put into words such tenuous correlated differences in texture and aspect come closer to the very essence of the species problem than do discrete, readily definable, characters. In his discussion of funda-

mental leaf form Velanosky (1905) said: "Die Blattform ent-
spricht nicht nur den biologischen Zwecken und morphologischen
sowie historischen Ursachen der beterffenden Pflanze, sondern
passt sich auch dem ganzen Baue und Stil der Pflanze harmonisch
an, wodurch die Pflanzen nicht selten ein prachtvolles Exterieur
gewinnen, welchem strenge, äesthetische Regeln zugrunde liegen."
One might adopt his generalization to the species problem and
say that species differences are harmoniously expressed through
the whole architecture of the plant, through the operation of
basic esthetic laws.

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WHITE-EYED MUTATION IN PHORMIA
REGINA MEIGEN

SEVERAL white-eyed individuals of *Phormia regina* Meigen
have been obtained in March, 1942, through the courtesy of the
Department of Animal Behavior of the American Museum of
Natural History, New York. The appearance of the white-eyed
mutants was noticed previously in the mass cultures kept by the
Department. A wild-type strain, with reddish-brown eyes, has
been established at the laboratory of the Bronx High School of
Science from a single female caught in New York City. The flies
were bred at room temperature, 70° to 72° F., sheltered from
direct sunlight; the development from egg to adult takes about
three weeks. Cane sugar, egg albumen and water were given to
the flies as food, and pieces of liver or lung were introduced into
the container on alternate days, for egg-laying. A true breeding

white-eyed strain was established without difficulty. The eye color in freshly hatched flies is pure white, but with age it acquires a yellowish or pinkish tinge.

In the first two experiments white-eyed females were crossed with red-eyed males, and red-eyed females with white-eyed males, respectively. The F_1 generation consisted of red-eyed flies of both sexes. Direct F_2 progenies were obtained; they included flies with red and with white eyes. In the third experiment F_1 females were back-crossed with white-eyed males; white and red-eyed flies appeared in the progeny.

The numerical relations are shown in Table I.

TABLE I

		First Experiment		Second Experiment		Third Experiment	
		Red ♂	White ♀	Red ♀	White ♂	Red ♀	White ♂
Females	....	482	169	732	247	108	120
Males	....	475	159	761	243	130	112
Total	Obs.	957	328	1493	490	238	232
	Exp.	964 ± 15.5	321 ± 15.5	1487 ± 19.3	496 ± 19.3	235 ± 10.8	235 ± 10.8

It is clear that the white-eyed condition is due to a recessive autosomal gene. In this respect the mutant in *Phormia regina* Meigen behaves like similar mutants in *Psychoda alternata* Say (Turner, 1923) and *Lucilia cuprina* Wied (Mackerras, 1933). White-eyed mutants are also known in several species of Drosophila, but in all these the mutant is sex-linked. Provided that the white-eyed mutants in Phormia, Lucilia and Drosophila are homologous, we have here either a case of translocation between the X-chromosome and one of the autosomes or a transfer of the sex-determining factors from chromosome to chromosome in the phylogeny.

I am grateful to Professor T. Dobzhansky, of Columbia University, for his guidance in connection with this experiment.

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WHITE SPOTTING IN THE FOX[1]

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THE foxes most generally raised in confinement for their furs are various color phases of the common red fox, which ranges over the northern parts of three continents —Europe, Asia and North America. It varies considerably throughout this range and systematists have at times divided the wild form into numerous species— Merriam proposed some ten or more in North America alone—but the intergradation and the apparent absence of any barrier to free interbreeding when opportunity offers would seem to make it more logical to consider the red fox a single holarctic species, *Vulpes vulpes* L. As is to be expected, mutants of the wild red are found from time to time in various localities. Aberrant pelts have long been known in the fur trade, and certain of these color variants, particularly the "black," have formed the basis of the fox raising industry, which has assumed such considerable proportions in the past forty years.

The red fox may be superficially described as in general of a rich fulvous color with black nose, ears and feet, and with under parts and tip of tail white. More careful examination, however, reveals that the white of the under parts is not a clear white nor is it uniform throughout. It starts at the nose, runs along the sides of the jaws and

[1] Paper from the Departments of Genetics (No. 304) and Veterinary Science, Wisconsin Agricultural Experiment Station, under a project of the University Fox and Mink Research Program.

as a band of varying width along the throat, breast and belly to the base of the tail, being narrowest at the mid-belly and wider on the throat and upper breast, and in lower belly and groin region. There may also be in some specimens some whitish on the back of the legs and even on the toes. The white areas as described are not sharply marked off from the red, but the margins tend to be indefinite, the red and white guard hairs intermingling. Furthermore, except as noted in a moment, the white is not clear, but for the most part has a dusky or "smoky" appearance due to dark underfur underlying the white guard hair. This same dusky underhair pervades all other parts of the body as well, except for the white tail-tip and occasionally small areas of variable size within the ventral white already mentioned. These smaller, pure white areas presumably correspond to the white spotting so commonly limited to the ventral side of many mammals when small in amount, but correlated with the generalized white-spotting pattern, as first clearly pointed out by Allen (1914). The more extensive dusky white on the ventral side of the red fox may be referred to as *albescence* when it is desired to distinguish it from the true white spotting. That there is a genetic difference between the two is clearly demonstrated when the one or more genes for the wild-type red drop out to give the ordinary "black" or "silver black" color phase, which is the standard ranch-raised fox of the industry. In the silver fox the red of the guard hairs and the albescent of the "white" areas is replaced by black. The silvering, which is caused by a light band on the guard hair in the red fox, remains in the black phase, giving the "standard silver." This silvering varies in amount in the wild red, and in silver foxes has been selected to produce the "quarter," "half," "three-quarter," "full silver" and paler grades of the trade. Only the pure white areas remain unchanged, irrespective of the color phase, and it is only this type of white spotting with which we are concerned in the present paper.

In most wild-caught red foxes there is no pure white on the under parts, although in some there may be small patches in the breast or inguinal regions; and the extent of the white tail tip is variable. Ranch-raised foxes, on the other hand, often have considerable pure white on the underline and on the feet, and it may extend up on the sides in the flank region. In others there may even be a white blaze on the face and a more or less complete white collar on the neck. This last condition is characteristic of two types now rather extensively raised, the platinum silver (or platina) and the white-marked silver.

Because of the cost of conducting breeding experiments with foxes, and particularly the high individual commercial value of some of the types, we have had to depend for our data on records obtainable from regular commercial ranches. We have made extensive use of such records only where we have been able to copy them at first hand and to consult with the breeder as to his standards of classification and similar points. We have confidence in the soundness of the findings on the main facts of inheritance presented but realize the data are not sufficient to establish fully some of the suggestions regarding the genetic relationship of the several characters discussed. It is hoped, however, that these suggestions may serve as a guide for further observation, and particularly for critical matings, on which we have at present only meager data or none at all.

The Platinum Silver Fox[2]

The "black" fox long had such a hold on the trade that other color phases were neglected. When they did occur they were usually disposed of summarily as indicating lack of purity in the standard stock. In 1938 Tuff, in a

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[2] This color phase has been variously known as Norwegian platina, platinum and Norwegian-type platinum. The official name in this country is platinum silver, though they are colloquially referred to simply as platinum. To avoid unnecessary repetition and to save space we shall employ the shorter form in this paper, so wherever "platinum" is used it is to be understood that platinum silver is indicated.

general discussion of the inheritance of color phases in foxes, described a new type being raised in Norway,
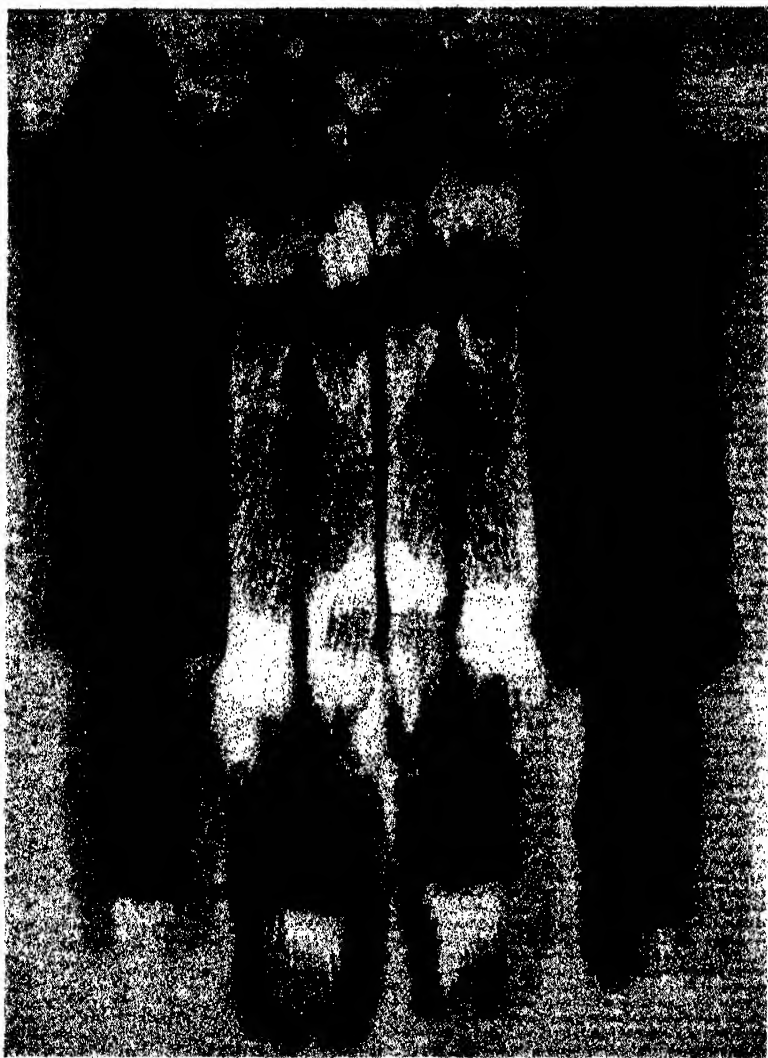
Fig. 1.   Two full silver pelts (outside) and two platinum silver pelts (middle), showing the distribution of white and dilution of the black in the latter.

which he referred to as "the platinum character in the silver fox"; and the following year Mohr and Tuff (1939)

presented a fuller account, together with a discussion of
the inheritance of the character. Since the appearance
of the Norwegian "platina" or platinum is rather fully
described and illustrated in the excellent report of these
authors, a more general description will be sufficient for
present purposes. The general color of the platinum is
lighter than that of the silver (Fig. 1), this being due not
only to the fact that there is a smaller pigmented area,
but also that the black appears dilute, with a grayish or
bluish tone. The actual shade varies greatly in different
individuals (Fig. 2), due in all likelihood to different
associated factors. Extremely light silvers often closely
resemble platinum, but any one familiar with them can
readily distinguish the two at all ages by the lighter tone
of the black. This is particularly evident on the ears,
which are deep black in the silver, whereas they may be
described as a medium gray or mouse-color in the plati-
num. The young pups are even easier to distinguish, as
the young platinums are dull bluish while the silver pups
are at first definitely black, only later acquiring the
silvering which makes them appear lighter.

The most characteristic thing about platinum is that it
is always accompanied by a considerable amount of white
spotting. Mohr and Tuff have well described it as fol-
lows: "White snout, a blaze along the nose and forehead
joining with a white collar around the neck. The breast
and a broad stripe on the belly are white and the same
is true of the legs and the distal part of the tail. The
size of the markings is somewhat variable, particularly
on the legs, and the white collar may be lacking in dark
individuals." As they add, in the white markings on
the nose and legs there are as a rule small black spots,
but these are not of the deep black color that they are
when they occur in silvers. As a matter of fact the
amount of white on the face may in some cases be so
small that the classification by phenotype might be doubt-
ful as an index of genotype if it were not for the other
characteristics of the platinum.
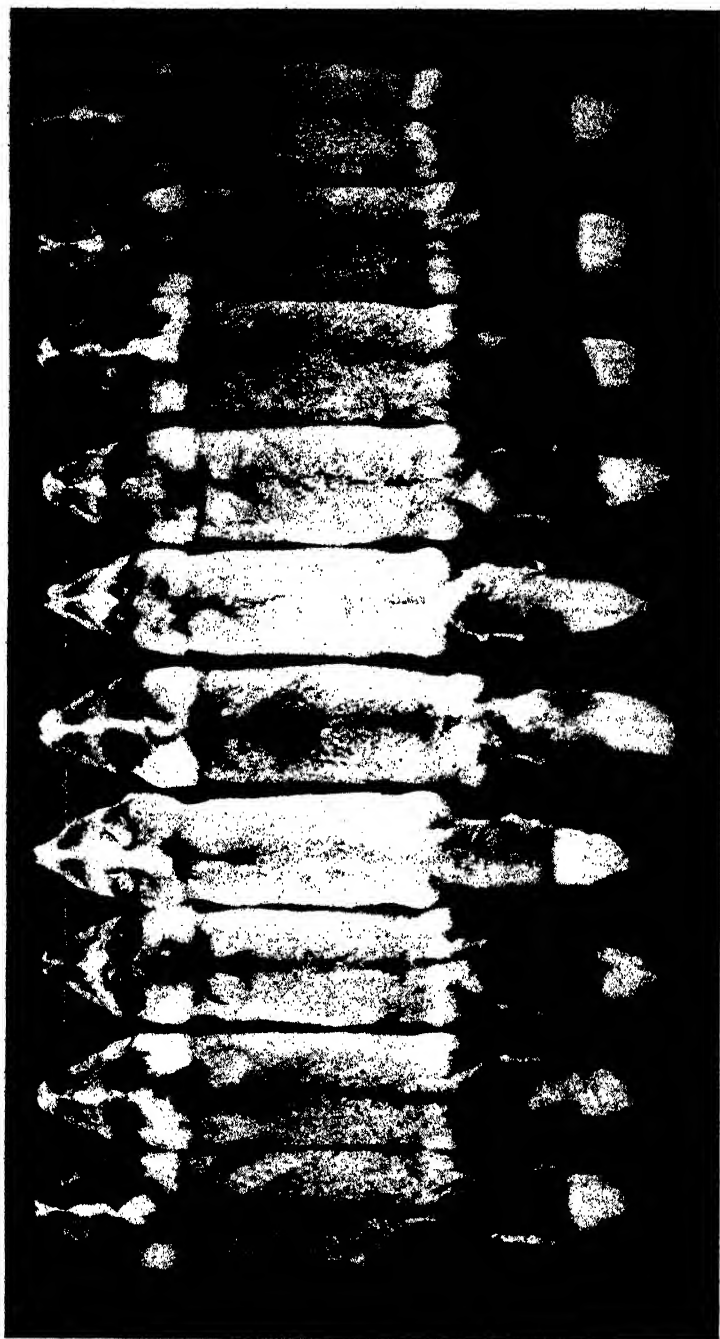
Fig. 2. Display of platinum silver pelts showing some of the variability in this color phase. More extreme examples occur, both with respect to amount of white and shade of color, than are here represented.

The Norwegian platinums are, for the most part at least, descended from a single mutant male known as "Mons," which appeared in 1933 in a litter of six pups from a long line of silver ancestors originally imported from Canada. Brager-Larsen (1940) says it was considered "a freak silver fox," which of course it really was. In the following year, 1934, one of the silver grandmothers of "Mons" gave birth by a different male to a litter of six pups, one of which appeared to be a typical platinum. This was a female, known as "Bergetta," and while it is not stated definitely it is presumed that her descendants have added to the Norwegian platinum stock. At the time Mohr and Tuff wrote, the "Mons" and the "Bergetta" descendants had not been interbred, so the authors cautiously refrained from stating whether the mutations were the same. It is interesting and possibly significant that these two mutants appeared in the same line of descent. There appears nothing else unusual in the pedigree of "Mons" except that "white markings" are noted as having occurred three or four generations back on the paternal side. There is relatively little inbreeding evident in five generations back, but this would not be necessary to bring out a dominant, which these appear to be. Two or three other somewhat similar and independently occurring mutations are mentioned. Little is known of these, but examination by Mohr and Tuff of one known as "7K" convinced them that it was distinct from the "Mons" type. It is possible this may have been of the white-face silver type discussed later in this paper.

Mohr and Tuff point out that the breeding records they had clearly indicate that platinum is an ordinary dominant, not sex-linked, and that the homozygous type was unknown. This was not surprising, considering that most of the platinums were from matings of platinum × silver. Such matings gave results approximating very closely the expected 1:1 ratio of platinum and silver offspring. The few available matings of platinum × platinum, however, gave more nearly a 2:1 ratio than the

3:1 ordinarily expected from the pairing of heterozygotes. To explain this the authors suggested the possibility that the dominant platinum gene may be lethal in homozygous condition. As will appear later, our findings support this suggestion.

According to Brager-Larsen (1940) Mr. Kjaer, who had bought ''Mons,'' in 1936 marketed the first platinum pelt at the Oslo sales for $200. The following year nine platinum pelts were offered and bid in for a Buenos Aires retailer, bringing as high as $500 for a single pelt and an average of $300. This favorable reception led to the formation of the Norwegian Platinum Fox Breeders' Association and a boom in prices of platinums and for matings of platinums to silvers ensued. It is stated that the usual price for matings was better than $1,000. The craze for platinum continued and it is reported that when, on January 22, 1940, a large part of the 1939 output from Norway was sold in New York, one skin brought a world record price of $11,000! The publicity resulting from this sale naturally concentrated the attention of American breeders on the new color phase and changed their attitude toward new colors in general. The Norwegian breeders placed an embargo on the exportation of platinum breeding stock, but it was soon apparent that there were already in North America at least three independent sources of mutations which were very similar to if not identical with each other and with the Norwegian platinum. It is true that any one thoroughly familiar with them can often tell to which strain a particular animal belongs but this is doubtless because of different accompanying minor factors which serve somewhat to characterize the different strains. For present purposes the description of the Norwegian platinum already given will suffice equally well for the American. Minor differences will be mentioned in connection with the strains, which have come to be generally known from the ranch on which each was developed and publicized and not necessarily by that on which the mutation first occurred.

The Corbin and Werth Strain

The mutant from which this strain was developed was born in 1937 on the Hillcrest Fox Ranch of Mr. Guy Corbin near Mount Horeb, Wisconsin. It was one of a litter of ordinary standard silver foxes. There was nothing unusual in the ancestry of this litter, and the only known inbreeding in the pedigree was that one individual appeared as both the maternal grandfather and the pater-

Fig. 3. Platinum silver fox. Photographed on Capitol Fox and Fur Ranch, Madison, Wis.

nal great-great-grandfather. The mutant was named "Hillcrest Chief," and is still alive (1942). In 1938 he was mated to a silver paternal aunt, named "Ruby," resulting in a litter of 2 platinum and 2 silver pups. This indicated that Hillcrest Chief is heterozygous, and subsequent breeding history has, as in the case of the Norwegian platinums, failed to disclose a homozygous platinum. Of the platinum pups, one was a male, which was given the name "Stagehand"; the other a female called "Sunbeam." The following year, 1939, Hillcrest Chief

was mated polygamously to five Corbin silver females and to one silver female belonging to the Capitol Fox and Fur Farm of Madison, Wisconsin. In the same year Stagehand was mated to a silver littermate and to a Capitol silver female. Sunbeam was mated to an unrelated silver male. These nine matings produced an aggregate of 22 platinum (14 males, 8 females) and 15 silver pups (5 male, 9 female, 1 ?). Six of the platinums were from the two Capitol females and this ranch bought also four of the other platinum males.

TABLE I

SUMMARY OF BREEDING RECORDS OF WERTH AND CORBIN PLATINUM SILVER AND SILVER FOXES (1938–1942)

Parents	Offspring			Total pups	No. of litters	Average litter size
	Platinum	Silver	White			
Silver × silver	0	1,215	0	1,215	267	4.6
Platinum × silver (Expected—1 : 1)	434	418	0	852	191	4.5
	426	426	0			
Platinum × platinum (Expected—2 : 1 : 1*)	12	8	3	23	11	2.1
	13.3	6.7	[6.7]			

* Calculated on the number of pups in the two viable classes.

Our association with the subsequent breeding of the platinums of this strain has been rather close, as Mr. John Werth and his son Anton, owners of the Capitol Fox and Fur Farm, have not only generously placed all their breeding records at our disposal but have made numerous matings suggested by us for their genetic interest and have given us access to their plant at all times (Fig. 3). This has afforded us opportunity for frequent consultation and for observation of the litters produced and their later development. Table I summarizes the available data on platinum matings in this strain. They include all the matings involving platinum on the Werth ranch and a few from Corbin's. The matings (191) of platinum × silver have produced 434 platinum and 418 silver pups, which is a reasonably close 1:1 ratio, and is in

accord with Mohr and Tuff's results for the Norwegian platinum (96 platinum to 88 silver).

Mohr and Tuff reported 22 platinum and 10 silver off-spring from mating of platinum × platinum and point out that this "approaches a 2:1 ratio instead of the expected 3:1." It was from this that they suggested that the homozygous platinum might be lethal. We have records on 11 litters from mating of platinum × platinum, with results as shown in the lower part of Table I. These results differ from those of Mohr and Tuff in that in addition to platinum and silver pups there were three that were *completely white*. This suggests that these white pups may represent the homozygous class, which usually dies at some early stage but from which an occasional pup may survive to birth or a little longer. For this reason these white individuals deserve further consideration.

The three white pups all came from the same platinum female mated successively in three years to the same platinum male.[3] The results of their litters is as follows:

> 1940, 1 platinum: 0 silver: 1 white;
> 1941, 0 platinum: 0 silver: 1 white;
> 1942, 1 platinum: 0 silver: 1 white.

Each of these white pups was entirely white with blue eyes. The one born in 1940 was not discovered until the nest box was examined when the litter was about three weeks old. It was alive and seemed to be doing well (Fig. 4) up to about five weeks of age, so was left with its mother. Then one morning all that was found was its tail; it had either died or been killed and eaten by the mother. The 1941 white pup lived to at least 28 days, but when it was to be taken out for "pilling" on the 30th day no trace of it was to be found. In view of the past experience, this female was watched closely at whelping time this year, for it was planned if there was another

[3] There is a possibility, though it is unlikely, that the mating was by a different platinum male in 1942. This, however, should not change the expectation.

white pup to remove it at once and attempt to raise it either by hand or with a cat as foster mother. The female whelped two pups on March 24, 1942, a platinum and a white. The white was only about half the size of the other and was apparently dead at birth; otherwise it appeared normal (Fig. 5).

The question may be asked, if the silver and the white represent the respective homozygotes, and platinum the
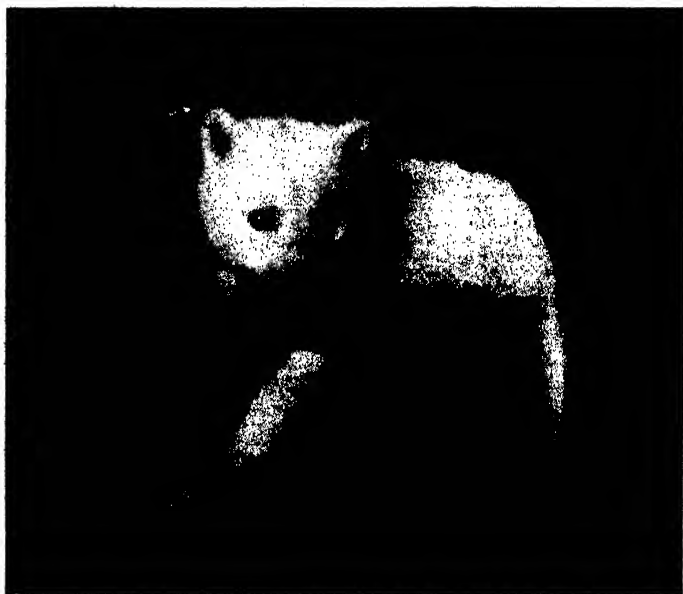
Fig. 4. Completely white pup, 24 days old, from platinum × platinum mating. Lived to about five weeks of age. Capitol Fox and Fur Ranch. This picture was first published in *American National Fur and Market Journal*, Vol. 18, No. 12, July, 1940.

heterozygote, why the ratio from platinum × platinum matings in general should be so far from 1 : 2 : 1. This may be in part due to chance—the available numbers are small—and probably a good deal to a differential death rate before the litters are observed. Pups that die in the nest box may be eaten by the mother and, as the litters on most ranches are usually not disturbed until they are about three weeks of age, any pup which died before that

time would be likely to be unrecorded. It is more than likely, for example, that if special care had not been taken to look for it the 1942 white pup would not have been seen at all. Furthermore, the fact that the white pups the two preceding years lived for an appreciable time would appear to be quite unusual, due perhaps to the individual physiology or behavior of this particular female. It is quite possible that the homozygous pups ordinarily die at some time during fetal development and that live birth is a rare event. The small average litter size (2.1) of the

Fig. 5. White pup from platinum × platinum mating. Dead when found. Capitol Fox and Fur Ranch.

platinum × platinum matings as compared with that from platinum × silver (4.5) is further evidence of some reproductive disturbance, and premature death seems a plausible explanation. The reduction in this case is more than could be accounted for by elimination of one fourth of the offspring, but the numbers are too small to make further speculation profitable. The platinum × silver average compares favorably with that of 4.6 in the silver × silver matings on the same ranch and during the same period. Kellogg (1941) has tabulated data from another Wisconsin ranch showing a total of 2,997 pups in 638 silver × silver matings. This is an average of 4.7 and indicates that not only are the Werth foxes represen-

tative in this respect but that mating of platinum to silver does not appreciably affect the litter size.[4]

Much variation is found among the platinum foxes with respect to the extensiveness of the white markings and the intensity of pigmentation or depth of shade on the colored portions of the pelage. The white markings, wherever they appear, may lack symmetry, white often extending up only one side of the neck rather than forming a complete collar or extending equally on both sides. The white tip of the tail is characteristically larger than in regular silvers, and there may be considerable white extending up on the flanks. Very light individuals do not necessarily have more extensive white markings, while dark ones may show much white. The darker platinums, however, appear to show more dark spotting in white areas than do the lighter ones. It is possible that some of this variability is non-genetic, but it is more probable that much of it is determined by factors in the silver foxes to which the platinums are bred.

For a conclusive test of whether platinums may ever be homozygous, a considerable number of platinums from platinum × platinum matings should be bred to silvers. This test is seldom made by breeders. They would like to secure a strain of true-breeding platinum and some have the idea that platinum offspring from two platinum parents should have the character "concentrated" and hence themselves produce a larger proportion of platinums. Not only has this not proved to be the case but it is not in accord with genetic expectation. Only one of the Werth platinums produced in this way in 1941 was tested and she produced both platinum and silver pups in 1942.

THE CODY STRAIN

A brief history of this strain of platinum foxes has been given by Mr. W. A. Granquist (1941). In 1937, according to his report, the mating on the Buffalo Bill

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[4] It should be kept in mind that these are not "ranch averages," as they apply only to certain classes and do not include infertile matings.

Fur Farm, at Cody, Wyoming, of a full silver standard male, M 125, with a three-fourths silver female, F. M. 38, gave a litter of four pups, three of which were normal silver and one, a male, was recorded as a pup as "almost white." This individual was named "America" and the Cody platinums are all descended from him. His ancestors had been on the farm since 1928 and were all standard silvers; furthermore, his parents produced four subsequent litters, all silvers. The breeding from "America," as compiled from the Granquist paper, is summarized in Table II. It will be noted that the essential equality of platinum and silver offspring corresponds to what has been found in other matings of this type.

THE LA FOREST STRAIN

The foxes which have come to be generally known as La Forest platinums are again descended from a single mutant individual. The strain has been developed by Dr. J. E. La Forest of Quebec, Canada, who has put on record a brief account of its origin and development (La Forest, 1941). In 1938 a male "freak" platinum was born on the ranch of a neighbor. The parents were registered silvers and had previously given four litters, all silvers. They were mated again in 1939, hoping for another platinum, but the litter was all silvers.

The platinum "freak" produced thirteen pups in 1939. The number of litters and the proportion of silvers to platinums are not given, but it is stated that there were "two full litters of pure platinums." One interesting mating was of the original platinum to a white-marked silver, that is, she was a silver with a white spot on the neck and also white spots between the front and between the hind legs. This mating produced two platinums, one silver with white markings like the mother, and one plain silver. The two platinum pups, a male and a female, were very largely white-marked, with hind legs full white and the white extending "all over the back, blue eyes, pink nose, pink feet and claws." The objective is to

obtain light, even color in the platinums, and it was a disappointment when ''the mating of an extra pale platinum male, with blue eyes and pink nose, to his half sister of the same quality and color . . . produced a litter of very nicely marked pups, but of very dark color with dark underfur, and of rather poor quality.'' This emphasizes again the considerable independence of these variable characters.

Judging from the appearance of the animals and from the breeding history it would seem probable that the La Forest platinums are genetically like the two other American strains and the Norwegian. Dr. La Forest, it is true, makes the general statement that his matings of

TABLE II

EARLY HISTORY OF PLATINUM BREEDING ON THE BUFFALO BILL FUR FARM, CODY, WYO., 1937-1939. (COMPILED FROM GRANQUIST, 1941)

Year	Mating	Offspring	
		Platinum	Silver
1937	"America" × silver full sister (F. A. 61)	2*	2
1938	"America" × silver full sister (F. A. 61)	1	2
	"America" × silver (F. A. 23)	1	2†
	"Young America" × silver (F. A. 141)	2	3
1939	"Young America" × silver (F. A. 23)	4	2
	A mating of "America" to a platinum female produced no pups		
	Total—platinum × silver	10	11

* One, a male, named "Young America"; other died.
† The number in this litter is not definitely stated, but the statement is made that it was a "similar litter" to the one immediately preceding.

platinum to platinum ''produced just as high and as healthy litters as any other matings of platinum to silver'' and that the percentage of platinum pups is about the same, but no data are presented. Of course, the genetic identity of these various platinums can not be definitely proven until controlled matings between the different strains can be made.

Platinum foxes with pink or spotted noses and with one or both eyes partly or wholly blue are not uncommon. This condition is quite obviously correlated to a considerable extent with the general amount of white on the animal and is not separately inherited.

WHITE-MARKED SILVER

The striking thing about the true platinum or platinum silver just discussed is that this color phase is always accompanied, genetically, at any rate, by white markings, which are usually more extensive than the small ventral spots and white tail tip of the silver. On the other hand, there are foxes which have the white blaze and other white markings similar to the platinum, but which otherwise are like ordinary silvers. These foxes are variously known among breeders as "white-marked silvers," "white-face silvers" and "ring-necks," depending on the

Fig. 6. White-marked silver fox. Photograph from Mr. Bruno Delsman, Hartland, Wis.

extent and location of the white (Fig. 6). Because of the resemblance of the white markings to those of the true platinums, the lighter colored specimens are often confused with platinums and pass as such, or they may be called "platinum-type silvers." There is no question that they sometimes are phenotypically very similar to platinum, the resemblance between the two types extending even to the frequent occurrence of blue eyes when there is a large amount of white (Fig. 7). But our examination of many animals of these types convinces us that the differences we previously outlined as distinguishing silver from platinum, particularly the shade of color
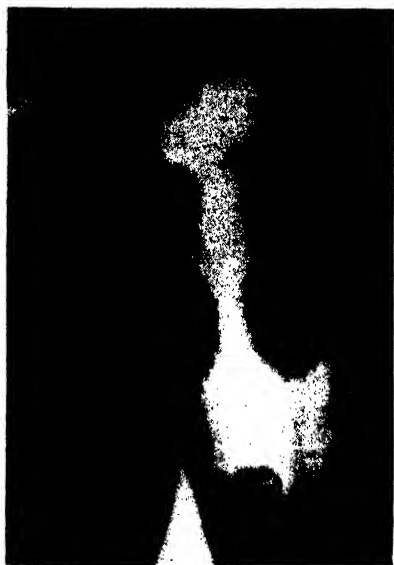
Fig. 7. White-marked silver fox with left eye blue, right eye normal. Photographed on ranch of Russell Colpitts, Salisbury, N. B.

in the young (Fig. 8) and of the ears in the adults, serve in this case to class these foxes as being really unusually light silvers on which the characteristic white-face mark-

Fig. 8. Two white-marked and two platinum silver 'pups about three weeks old, showing contrast at that age. Photographed on Capitol Fox and Fur Ranch.

ings have been superimposed. Combined with this may be another characteristic consisting of white hairs, not barred, sprinkled through the pelt, similar to the "silvering" sometimes found in rabbits and mice. To distinguish it from the silver character in foxes, we have termed it roaning. It is variable in amount but apparently increases with age. When present in considerable degree it lightens the pelt appreciably and increases the superficial resemblance to true platinum. Whether roaning is entirely independent of white face in its inheritance is not known.

Regarding the origin of white marking, Mr. Harry J. La Due (1937) says: "White spotting in silver black foxes occurred early in the industry but most breeders quickly eliminated such animals from their herds. They were first noted in Canada and then in some of the old-time herds around Muskegon, Michigan. The wide distribution of breeding stock from these old herds has caused the appearance of white spotted foxes in many places in the United States." He elsewhere (1929) speaks of these Muskegon foxes as being "spotted like Boston Terriers."

It is probable that this mutation has occurred independently a number of times, but information as to its independent origins is scanty. The fact that different strains are recognized, such as the Colpitts, the McNeil, the Garvey or the Holman white-faces, is not evidence that each has descended from a separate mutation, but certainly some of them have. The Colpitts mutation is said to have originated in Calgary in 1928 (Avery, 1941).

The Springborn or Kaempf foxes apparently arose from an independent mutation. According to Mr. W. J. Boston (1942), the Wausau Silver Fox Ranch, Inc., in 1929 sold some silver foxes to Ed. Springborn of Shawano, Wisconsin. The following year Mr. Springborn complained that one pair had thrown, in a litter of four, "a pup that looked like a collie dog." This "freak" was taken back by the Wausau ranch and was

registered in the American National Fox and Fur Breeders Association under the name "Wausau Sport E," and tattoed W.W.–E 196. The interesting thing about this individual was that his coat was "sprinkled quite profusely with white hairs." The white-face character seemed to breed as it does in other cases, but it is stated that part of the white-face progeny had this characteristic of acquiring white hairs and lightening up when about two years of age, whereas the others did not; nor did the plain silver segregates develop this character, which presumably is the roaning previously mentioned. The individuals with the character are said to develop into a beautiful pastel shade of blue gray and are given the fancy name of "pastel platina silvers."

Breeding of White-marked Silver

A significant thing in the breeding of white-marked silvers is that, as is the case with platinum, apparently there is no evidence that they are ever homozygous for the character—matings of white-marked to plain silver always produce these two types in approximately equal numbers. While this is the regular experience of breeders, our definite and, we believe, reliable records in support of it are limited to a few sources. Those cases in which we have been able to copy down the litter-by-litter records are summarized in section (a) of Table III. The grand total of 282 white-faces to 241 without white faces is reasonably close to the expected equality if the white-face parents were all heterozygous. This is, of course, only indirect evidence that the homozygous condition is non-existent among breeding animals, but two other bits of evidence point towards the same conclusion.

It will be recalled that the mating of platinum to platinum resulted in greatly reduced litter size, which was ascribed to prenatal or neonatal lethality of the expected homozygous "platinum" pups. Section (b) of Table III gives indication of a similar situation in the mating of white-marked × white-marked, the resulting 183 white-

marked to 79 silver pups being nearer a 2:1 than a 3:1 ratio. Even more significant is the reduction in average litter size, the drop from 4.6 to 3.3 corresponding almost exactly to the expected if one fourth of the offspring are eliminated. The conformity of these results in the data from different sources is striking.

Still further evidence of the similar behavior of white-face when associated with silver and when associated with platinum is furnished by the appearance of pure white

TABLE III

SUMMARY OF MATINGS OF WHITE-MARKED TO STANDARD SILVER* AND WHITE-MARKED TO WHITE-MARKED FOXES

Source	Offspring		Total pups	Total litters	Average litter size
	White-marked	Stand-ard			
(a) *White-marked* × *standard silver*					
McIlquham and McGill (1941)	20	14	34	9	3.8
Fred C. Beck (1941–42)	86	78	164	36	4.6
Sanford Colpitts† (1941)	157	132	289	60	4.7
Capitol Fox and Fur Farm (1942)	19	17	36	8	4.5
Total	282	241	523	113	4.6
(Expected—1 : 1)	261.5	261.5			
(b) *White-marked* × *white-marked*					
Sanford Colpitts (1941)	164‡	72	236	71	3.3
Fred C. Beck (1941–42)	19	7	26	8	3.3
Total	183	79	262	79	3.3
(Expected—2 : 1)	174.6	87.3			

* Some other color types, such as "pearl platinum," have been included since they are independent of white marking and in relation to it behave the same as standard silver.
† There may be included here a few matings of Strain B white-marked, to be discussed later, but this should not change the ratio expected.
‡ Three of these pups were classed as white-eared; discussed in a later section.

pups in matings of white-marked to white-marked as shown in the first section of Table VI. These two matings are not included in Table III because they are selected from the records on other ranches. We had opportunity to examine the one white pup on the Russell Colpitts ranch as it had been preserved in alcohol and it appeared in all respects similar to those produced in the platinum matings (Table I and Fig. 3). The four white pups in one litter on the Curtis-Davis ranch happened to

be found because the mother had trouble in whelping. All four were naturally bobtailed.

Relation of White-face (White-marked) Silver to Platinum Silver

It has been shown that platinum and white-face are practically identical phenotypically with respect to the white markings. They also correspond in that both apparently exist only as heterozygotes, that reduced litter size in heterozygote $\times$ heterozygote matings suggests homozygote lethality, and that completely white pups, dead at birth or soon after, are occasionally produced in both types. The only constant difference is that the black pigment appears to be more dilute in the platinum. The question naturally arises as to whether these are two separate and independent mutations, involving different loci on the same or on different chromosomes or whether white-face and platinum are alleles and both allelic to silver (non-white-marked). Unfortunately the reliable evidence bearing on this point is scanty but seems of sufficient interest to present (Table IV). The genes for platinum and white-face might be symbolized as P and W, respectively. If they are independent, with free segregation, the mating of platinum to white-face would be expected to give offspring in the ratio of 2 platinum: 1 white-face: 1 silver. This is on the assumption that the genotype with both P and W is not lethal except when one or the other is homozygous. On the other hand, if white-face and platinum are allelic, in the order of dominance $w^+ \rightarrow W \rightarrow W^p$, the expected ratio from the above mating would be 1 platinum: 1 white-face: 1 silver, the WW^p combination being lethal. The results from three such matings, 7:6:5, approach this latter ratio but are insufficient to be critical.

The real test of the two hypotheses lies, of course, in the breeding of the platinum offspring from the platinum $\times$ white-face cross to silvers. On the assumption of independence, half the platinum offspring when mated to

silver should produce all three classes of pups in the ratio of 2 platinum: 1 white-face: 1 silver, and the other half only platinums and silvers, in equal proportions. Assuming triple alleles, all the platinums from the first cross should produce only platinum and silver offspring, and in equal numbers. We have definite record of only one animal so tested—a male produced on the Werth ranch in 1941. Bred to three silver females he produced 9 platinum, 10 silver and no white-face.

TABLE IV

MATINGS INVOLVING PLATINUM AND WHITE-FACE SILVER

Source	Offspring			Total pups	No. of litters	Average litter size
	Platinum	White-face	Silver			
Platinum × white-face						
Ekstrom (1942; *teste* Werth)	2	4	1			
Werth (1941)	2	0	3			
Fred C. Beck (1942)	3	2	1			
Total	7	6	5	18	3	6
(Expected on multiple allele hypothesis— 1:1:1)	6	6	6			
(Expected on independent gene hypothesis— 2:1:1)	9	4.5	4.5			
Platinum ♂ (from Werth mating above) × 3 silver ♀♀						
Werth (1942)	3	0	4			
	4	0	3			
	2	0	3			
Total	9	0	10	19	3	6.2

The litter size in all these matings is surprisingly high even if no genetic lethal were concerned. There is certainly no obvious reason why it should be above that of ordinary silver × silver matings. Moreover, in the matings of platinum × white-face, death of one fourth of the pups would be expected on the multiple allelic interpretation, and in so far as the litter size in these three matings has any significance it would seem to favor the idea of independence. The number of litters concerned, however, is too small to carry much weight.

Without decisive evidence either way we are inclined to adopt provisionally the multiple allelic interpretation as being on the whole the simpler. Independence would

involve two loci, at one of which mutation occurred to produce white-face and at the other a mutation producing both white-face and dilution of black, giving the platinum. The allelic interpretation would furnish a plausible explanation of the fact that platinum is always accompanied by white-face—the white-face mutation is a first step and platinum a further one, involving dilution. Without

1. SILVER $(\omega^+\omega^+)$

2. WHITE-MARKED SILVER $(W\,\omega^+)$

3. POTENTIALLY WHITE $(W\,W,\ \text{LETHAL})$

4. PLATINUM SILVER (WHITE-MARKED, $W^P\omega^+$)

5. POTENTIALLY WHITE $(W^P W^P,\ \text{LETHAL})$

6. PRESUMABLY POTENTIALLY WHITE AND LETHAL $(W^P W)$
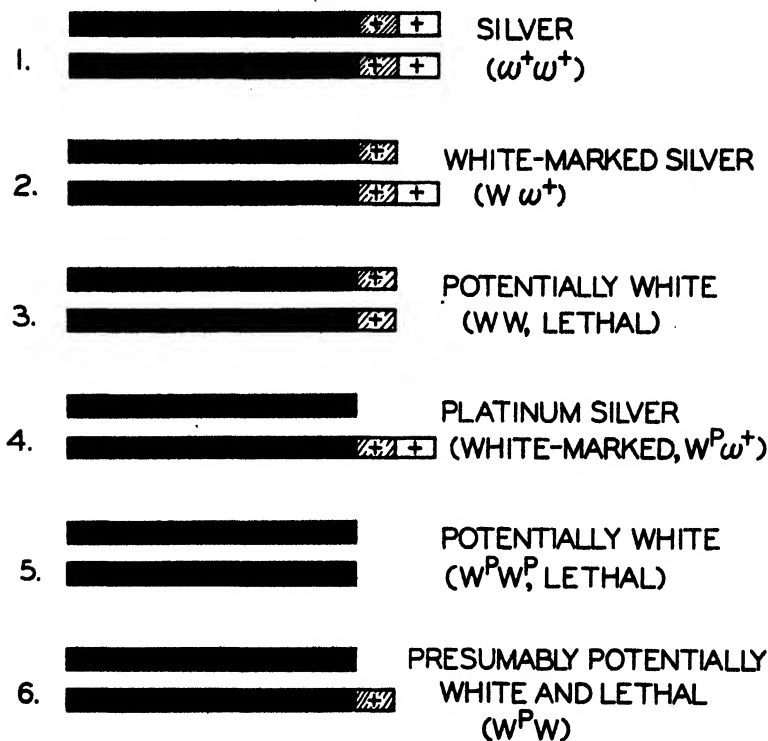
Fig. 9. Diagrammatic representation of chromosome types on deficiency hypothesis. Symbols in parentheses indicate the phenotypic effect of the assumed deficiency.

commitment as to the actual mechanics involved, this might be represented diagrammatically as a progressive deficiency (Fig. 9). As depicted, the bars at the top (1) represent the normal wild-type chromosomes, producing a silver fox, with a normal gene for self-color in the right-hand terminal section and one for intense black pigment in the section next to it. A unit break at the right end

results in white-face (2) and is lethal if homozygous (3). A second break (4) produces platinum, and of course white-face, and this would also be lethal when homozygous (5). In every case, for a fox to be viable one of the pairs of chromosomes must be complete. These assumptions are sufficient to account for all the known breeding facts. On this interpretation a deficiency (absence) produces a dominant effect; many cases of this sort are known.

Two Factors for White-marking

On the Sanford Colpitts ranch we were shown a striking type of white-marked fox we had not observed elsewhere,

Fig. 10. ''White-ear'' fox on the Sanford Colpitts ranch, Salisbury, N. B. Note the extension of white up the ear producing black borders and white tip.

though we had seen occasional individuals somewhat approaching it phenotypically. These animals not only have the white face and the neck ring well marked, but in addition the white extends up on the ears, particularly up the middle, producing a black border on each side and a conspicuous white tip (Fig. 10). There is considerable variation, as would·be expected; the white is often unsymmetrical and may in some cases involve only one ear.

In general, however, there is little difficulty in distinguishing these "white-ears" from other white-marked foxes.

We were told that the "white-ear" character appeared from crossing two strains of white-marked silvers from different sources. These two strains are in general very similar, though as they occur on that ranch one can usually be distinguished by a characteristic triangular white marking on the neck at the base of the skull. This area is usually not white in other white-marked silvers, or in platinums, unless it is included as a part of a complete or incomplete collar. Both these strains will be referred to collectively as white-marked, but since they appear to be fairly distinct and to have a complementary action, increasing the amount of white and producing white-ears when crossed, the regular type may be designated Strain A and that characterized by the triangular spot as Strain B. For brevity, as heretofore, the plain silvers (without white markings) will be referred to simply as silvers, although, of course, all these types show silvering in the pelt.

The breeding results with white-ear shown in Table V support strongly the breeders' idea that white-ear is different genetically from white-marked. If it were due to the same gene as white-marked, influenced perhaps by modifiers for extension of white, mating of white-ear to silver should give a $1:1$ ratio when white-ear and white-marked offspring are combined. The numbers obtained, 196 to 89, differ significantly from equality and suggest that Strain B may depend upon a different gene for white-face, similar to W in its phenotypic manifestation and in being lethal when homozygous, but located at some distance from it, presumably on a different chromosome. This Strain B gene may be designated as W_{II} and its normal allele as w^+_{II}. The further assumption is necessary that W and W_{II} have a supplementary effect such that when both are present the white is considerably extended, running up on the ears, and producing the

white-ear type, which would perforce always be of geno-
type $W w^+ W_{II} w^+_{II}$.

The results of the available matings shown in Table V
may now be examined on the basis of these assumptions.
Mating of white-ear to silver should by formula produce
1 white-ear: 2 white-marked: 1 silver. The obtained
55:141:89 deviates considerably from this expectation
but still has a probability of .05–.01 that the deviation is
due to chance. No deaths from genetic cause are ex-
pected in this cross and the average litter size of 4.7 indi-
cates that such have not occurred.

In the mating of white-ear × white-marked, shown in
the lower part of Table V, expectation is that genetic

TABLE V

MATINGS INVOLVING STRAIN A AND STRAIN B WHITE-FACE (DATA FROM
SANFORD COLPITTS RANCH, 1941 SEASON)

| Parents | Offspring | | | Total pups | No. of matings | Average litter size |
	White-ear	White-marked	Silver			
White-ear × silver	55	141	89	285	60	4.7
		196	89			
(Expected— 1:2:1)	71.25	142.5	71.25			
(Expected— 1:1)		142.5	142.5			
White-ear × white-marked	38	62	18	118	34	3.5
(Expected— 2:3:1)	39.3	59.0	19.7			

lethality would remove one fourth of the pups and that
the remainder should appear in the proportions of 2
white-ear: 3 white-marked: 1 silver. The obtained result
of 38:62:18 respectively in these classes is uncannily
close to this expectation, but the phenotypic classification
as we copied it down had already been made by the
breeder on the young pups without any thought of genetic
theory. Equally striking is the fact that the average lit-
ter size is reduced almost exactly one fourth from that
of the white-ear × silver (4.7 to 3.5).

Mention was made in the discussion of platinum that
some individuals have one or both eyes partly or wholly

blue, particularly when there is a large amount of white on the face. The same is true of the white-marked silvers and blue eyes are even more prevalent in the white-ear foxes, in which there is usually an even greater extension of white. Furthermore, the white-ear foxes as a group appear to be more nervous than the other classes; this is manifested in greater activity and sometimes by a habit of throwing the head backward and to one side. A few individuals exhibited circus movements, somewhat like those of the waltzing mouse.

WHITE PUPS IN WHITE-MARKED AND WHITE-EAR MATINGS

Our records of completely white pups from platinum × platinum matings were presented in Table I and discussed in that section. In Table VI are presented data of five litters involving white-marked and white-ear which produced a total of nine white pups similar to those from the platinum matings, except that none of these was alive when found.[5] It was the opinion, however, that some of them at least were alive at birth as they had sand inside the mouth, whereas it probably would not have got in so far if they had been dead.

The five white pups from the white-marked × white-marked matings have already been discussed (p. 309). The numbers in the other matings are too small to make a discussion of the ratios profitable further than to point out that on the basis of the genetic interpretation suggested lethality would be expected in both types of matings. In the white-ear × white-marked mating one fourth of the pups would be expected to fall in the "prenatal lethal or white pup" class, while in mating of white-

[5] In *Canadian Silver Fox and Fur* for July, 1942 (Vol. 8, No. 7, p. 5), is the picture of a white pup from the mating together of "two ring neck animals" which were half-sister and half-brother. The pup lived to five weeks of age and appears from the photograph and description in all respects similar to the white pups reported in this paper. This case occurred on the ranch of Mr. Lowell W. Hancock, of Prince Edward Island, and was reported by Dr. C. K. Gunn, director of the Dominion Experimental Fox Ranch.

ear $\times$ white-ear the expectation would be nearly half (seven sixteenths) in that category.

Mohr and Tuff stated that the Norwegian platinum character is not sex-linked. We have found the same to be true in this country; and this applies as well to white-marked. The records do not include the degree or extent of the white markings, hence we have not been able to grade individuals and classify them with respect to sex. It is our impression, however, and we have encountered

TABLE VI

SELECTED MATINGS INVOLVING WHITE-MARKED AND WHITE-EAR WHICH PRODUCED COMPLETELY WHITE PUPS

Parents	Offspring				Total pups	No. of litters	Average litter size
	White-ear	White-marked	Silver	White			
White-marked × white-marked	0*	2	1	1	4		
	0†	1	0	4§	5		
Total	0	3	1	5	9	2	4.5
White-ear × white-marked	1‡	2	1	1	5	1	5.0
White-ear × white-ear	0‡	0	0	2			
	1†	1	0	1			
Total	1	1	0	3	5	2	2.5

* Russell Colpitts Ranch.
† Howard Colpitts Ranch.
‡ Curtis-Davis Ranch.
§ All four naturally bobtailed.

no opinion to the contrary among breeders, that the white-face character is expressed equally in both sexes.

Throughout the records there appears to be a tendency for an excess in the classes having white markings over the plain silvers. This is true both in the platinum (Table I) and the white-marked (Table II). In the case of the latter it might conceivably be due to faulty classification of some of the borderline phenotypes, but this explanation would not apply to the platinums. At present we have no other explanation to offer.

SUMMARY

(1) Platinum silver, the platinum color phase in silver foxes, first brought to attention by Norwegian breeders,

appears to have arisen independently by mutation in at least three instances in the United States and Canada.

(2) The platinum character is characterized by a dilution in tone of the black pigment in the coat, accompanied by white markings, particularly on the face and neck, which are usually fairly extensive.

(3) Platinum behaves in inheritance as a dominant which is lethal when homozygous. This is supported by the fact that only heterozygous individuals are known and the further fact that the litter size is reduced in platinum by platinum matings. Completely white pups which are dead at birth or die shortly after are occasionally found. These are taken to represent individuals of the homozygous dominant class which have survived longer than is usual or by chance have been found.

(4) A phenotypically similar white-face mutation is found in the silver fox. This differs from platinum in that the tone of the black is intense, rather than dull, particularly on the ears, elbows and other places where it is not so much intermixed with white or white-banded hairs. This difference in intensity of the black is particularly diagnostic in the young pups, the platinums at that stage being bluish, the white-face silvers definitely black.

(5) Inheritance of white-face is similar to that of platinum in that the dominant homozygote is lethal, litters from heterozygote $\times$ heterozygote matings are reduced in size, and completely white pups are occasionally found in such matings.

(6) The data are insufficient to settle definitely whether the genes determining them are independent or allelic. The latter hypothesis is adopted provisionally as being the simpler, and the symbols w^+, W and W^p are adopted for standard silver, white-face and platinum, respectively, in order of dominance. The white-faces in this allelic series are denominated Strain A.

(7) It is suggested that the relation of white-face and platinum to silver might be explained by a progressive chromosome deficiency.

(8) Another strain of white-faced, or white-marked, foxes is very similar to Strain A in phenotypic appearance and seems again to have the same characteristics of
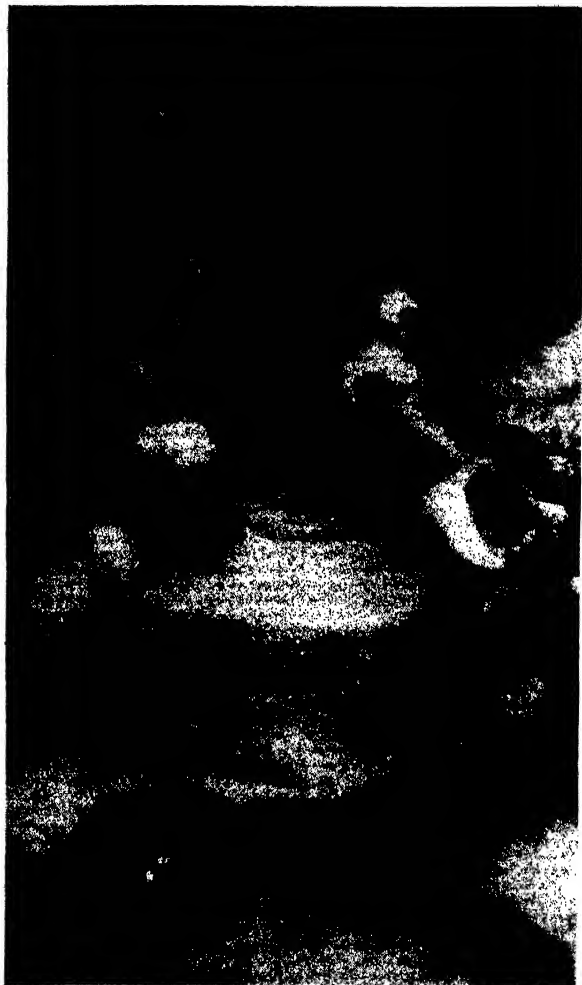
Fig. 11. Display of white-ear and white-marked foxes at a breeders' show. Photograph supplied by the Colpitts ranches, Salisbury, N. B.

inheritance. The breeding data, however, indicate that it is not an allele of Strain A, but that the gene, or possibly deficiency, responsible for it involves a different chromosome pair. This second strain we have called Strain B.

(9) Strain A and Strain B appear to be supplemental in their action, as foxes carrying genes for both have the white markings of the face and neck considerably extended. Commonly the white extends up on the ears in a characteristic fashion which has led to naming this type of foxes "white-ears."

(10) Blue eyes occasionally occur in each of the types of foxes discussed in this paper. The blue may invade the iris of one or both eyes (*heterochromia iridis*) or one or both eyes may be completely blue. In general, the greater the extent of white on the face the greater the likelihood of blue in the eyes. For this reason blue eyes occur with greater frequency in the white-ear type than in the others. The white-ear foxes also appear to be of a more nervous temperament as indicated by their actions.

(11) Platinum and white-marked are not sex-linked and appear to have equal phenotypic expression in both sexes.

fortunate to have this opportunity at our door, so to speak. We have obtained extensive and valuable records, which have obviously been taken with care, from Mr. Fred C. Beck of Thiensville, Wisconsin, from Mr. Tom McGill of McIlquham and McGill of Packenham, Ontario, and from Mr. Sanford Colpitts of Salisbury, New Brunswick. In this connection we should mention also Messrs. Russell and Howard Colpitts and the Curtis-Davis ranch, also of Salisbury, for selected records relating particularly to the completely white pups. Finally, we should be ungrateful if we did not mention the unflagging interest and help of Dr. Walter Wisnicky, director of the University Fur Animal Research Project.

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THE HERBARIUM IN MODERN SYSTEMATICS[1]

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My favorite dictionary informs me that a herbarium is "a systematized collection of preserved plants." Perhaps wisely, it has left to the individual the task of defining for himself the function of such a collection. Standing to-day at what surely must be a crossroads in the history of the world it is perhaps wisdom on our part to ask what rôle the scientific museum—and especially the herbarium—should play in the future development of our science and its relation to the advancement of the world's culture. It is particularly fitting that we discuss these matters on this occasion.

To-day there is some unrest among certain biologists who hold that there is scarcely any need for the maintenance of herbaria—that they are little else than expensive luxuries, club-houses for doddering systematists; that they have but little function within the body of biological science and should be eliminated. Some of these even say that the systematist is so far out of touch with the actualities that the species about which he so confidently writes do not really exist—that they are no more than subjectively defined population segments based upon a few specimens lying in museum cases and therefore temporary concepts existing only in the mind of man. To these critics the science of biological systematics is a hollow shell of long-outmoded concepts which had better be forgotten. For those who may be curious, it can be said that there are many who think otherwise. And I am one of them. It is my opinion that, although often genetically and morphologically complex, species are entities capable of being subjected to exact analyses of various sorts—otherwise this would never have been written. Yet there

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[1] An address delivered at the dedication of the M. A. Chrysler Herbarium, Rutgers University, April 16, 1942.

are those among us who do look upon species solely as collections of dried and dusty museum specimens to be juggled hither and thither—taken from a named pigeon-hole and slipped into one of another name—because of an indecision as to what these names should represent.

There are some, wandering as in a dark and trackless forest, who have sought solace from the pain of this indecision in a manipulation of nomenclatural categories—crying aloud that by so doing they were following the path which, ultimately, would lead us to the truth. Following their fancies, they explored each nook and cranny, setting up systems for the replacing of each scre and fallen leaf upon its twig and for following each worm-hole to its worm. But each succeeding year the trees cast down additional leaves and the worms left more holes behind them, so that those whose business it was to classify things came to think only of dead leaves and worm-holes.

They designed special cases in which to save samples of their treasures and published interminable volumes in which they argued as to who among them know most about dead leaves and worm-holes. Until finally it became necessary to set up a system of rules, not only as to what one might, or might not write about dead leaves and worm-holes, but as to the manner in which it was to be published. Thus, the system of classification—the system of names—became a Sacred Cow to be worshipped for itself. And many forgot about the green growing forest which produced the leaves and the burrowing worms which made the holes.

I shall not dispute with those, lost in a system of classification based on rules, who wish to quibble over the nature of species. They are only trying to extract the milk of wisdom from their Sacred Cow, knowing not that long since she has run dry. The least they can do is to lead her into greener pastures and leave her there until such time as her udder is replenished. They should return to a consideration of living organisms.

But, you may ask, if the systematist is to return to a study of living organisms, is this not proof that the herbarium is an outmoded institution? It is a reasonable question and should be answered.

Man early became aware of the individual objects of his environment. He also learned that objects of similar appearance had similar uses. Therefore, he set up a system of classification—a system of names—for greater ease in the retention and communication of concepts and information. The invention of the herbarium with its labeled specimens was a primary step forward in the further stabilization of these concepts and names as applied to plants. There is a tremendous social functionality to these names, for they are fundamental in so many of the arts, sciences and industries, being necessary for the intercommunication of basic information. It is therefore legitimate that we make some inquiry into the methods whereby a greater functional stability may be achieved and the part which the herbarium can play in its realization.

Methods, Concepts and Nomenclatural Stability

I was once chatting with a friend who is an advanced amateur field naturalist and he remarked that to him the most remarkable thing about taxonomy was the fact that two men—and reputed authorities—could ''burrow through the same pile of specimens and one emerge with five species and the other with seven species and a half dozen varieties.'' Had one wished to discourage him in his avocation, examples of considerably greater differences of opinion could have been pointed out.

As was intimated, there is a functionality to this system of names, otherwise it would long since have been discarded. But if it is to be genuinely useful there must be some continuity, otherwise chaos results. Sensing this need, the leaders of our science from time to time have sought to establish sets of rules whereby stability of concept might be achieved. Linnaeus had his set of princi-

ples. And De Candolle, who was the real father of our present epoch, outlined his system in considerable detail. This was followed by various schools of thought, among them the proponents of the so-called Berlin and Kew rules. There were the Paris and Vienna codes; these to be followed by the American code and the more recent sets of International Rules of Botanical Nomenclature. For those who are so inclined it is an interesting and revealing diversion to bring these documents together and compare them.

Although the avowed purpose of these systems and codes was to bring stability to the nomenclature of our science, where is it? As an example, Deam (1941) has pointed out that in the first year following the publication of his Flora of Indiana it was necessary—in attempting to follow only a portion of the current and valid treatments of others—to change one per cent. of the names. Does this mean that in the not too distant future the good people of Indiana, although probably blessed with much the same floristic elements as to-day, will be using an entirely different set of names?

Nevertheless, as much as we may desire nomenclatural stability, we can not expect that the names of organisms will remain completely static. We may, I trust, disregard for the moment the impractical and scientifically useless activities of those among us who are primarily concerned with the deft art of nomenclatural juggling. Yet there is need that we keep clearly in mind that the names we use can represent no more than the state of our current understanding of those biological units we call genera and species.

Therefore, let us recall that there are three quite separate phases in systematics. The first is the *Exploratory Study*, wherein but few specimens are available. It is these few and scattered examples upon which our primary concepts of species are based. Yet these types are often mere scraps or, because of the accidents of exploration, were collected at the margin of a species' range and are

therefore quite likely to be atypical. Specimens accumulate in our museums and the time is eventually ripe for the second phase: an extended survey of the material. This is the *Systematic Study,* resulting in what is often called ''the monograph.'' Here, confronted with considerable diversity and too often with numerous intergradient individuals, the student must determine as best he can the modulus of variability which is to be permitted in his population units; he must delimit his species. Unfortunately, in this chore, the rules of nomenclature are of no help. They only warn the student that every individual must belong to a species, every species to a genus and every genus to a family. There must be no loose ends in this system, for every specimen must be annotated and neatly filed in its pigeon-hole. But since subjective methods only are available in these phases of the work—and these based upon personal concepts—there is no guarantee that any two systematists will agree on group limits. It is for this reason that systematics is sometimes called an art rather than a science. The wonder of it is that there is any agreement.

Fortunately, through the maze of disputation which surrounds much of our work in systematics, a clear path is being opened. It is perhaps the ''New Systematics'' which some have prophesied. Yet I find in it nothing really new; it is only systematics at work with the tools which lately have been made available to it by other and increasingly more closely allied fields of biological endeavor. Because of its broader scope, this new phase into which systematics is adventuring may be called *Biosystematy,* for it no longer looks upon species as piles of named specimens neatly filed in museum cases, but as populations of living organisms. Any one who has carefully read the recent work on ''Genetics and the Origin of Species'' by Dobzhansky (1941) can not help but sense the dynamic nature of populations and realize the need to reinterpret our taxonomic units from this standpoint.

There are perhaps some who envision a stabilization of nomenclature in the very near future. I would consider

myself indeed fortunate if I could announce that, ultimately, the biosystematic phase of our science would lead to complete stability. But this privilege is denied me. It is perhaps best that we accept as a principle that our present tools of research will be further perfected and that new ones will yet be discovered. With these will come additional knowledge and, as a consequence, new concepts. If our nomenclature were to be stabilized by fiat—as some have suggested—we would first be required to bring our concepts to a completely static condition. If this were done, systematics would no longer be a science. It would be a filing system capable of being run by clerks, a condition in which it came perilously close to finding itself until revitalized by an application of the pertinent discoveries in other fields of biology in the last quarter century.

Nevertheless, although we can never expect a complete stability of nomenclature in biological systematics (for living populations will continue to erupt new biotypes), something approximating it—a practical stability—can be reached. It is my opinion that this can be achieved through biosystematy. It is in this phase of our work that we can learn the true nature of the population units with which we are dealing and thus define their limits in a more precise manner.

There are perhaps some who would object to the viewpoint that there is need that we have a common concept, that to do so would take away their jealously guarded privilege of personal interpretation, that it would put a stop to their "researches." They are at once both right and wrong. Even a preliminary biosystematic analysis of some group might stop much of their nomenclatural tinkering with it, but it would not stop work on the nature of its species. As a perhaps analogous example from another field, the case of so common a substance as water might be cited.

The early history of the concepts of the composition of this substance will not be rehearsed. But there ulti-

mately came a time when water was understood to be a chemical association of hydrogen and oxygen in certain proportions. From the practical standpoint, the water molecule was chemically defined. But this did not stop research on the nature of water.

It was learned how water molecules could enter into chemical combination with those of other substances (just as the individuals of different species sometimes combine their heredities to produce yet other species). The reactions of water in different environments was studied—its reactions at high and low temperatures and at different pressures, as well as under the influence of other conditions. It was even found to be no simple substance, but to contain a series of isotopes, in part the "heavy water" of our popular literature. But the point is, to the chemist, it is still water—and still composed only of hydrogen and oxygen. In spite of its known solvent powers, no one has yet proposed that, since water and sodium chloride are mutually soluble, water should be chemically redefined so as to include a part of the sodium chloride molecule. It is therefore likely that biosystematy, by defining the genetical limits of the population units we call species, can ultimately bring to our science a nomenclatural stability closely akin to the chemist's concept of the term "water."

However, before this stability can be achieved there must be a greater correlation of our scientific endeavors— there must be a concerted attack on the problem from as many angles as possible, using all the research tools which are available. It is therefore fitting that we briefly discuss the problems of correlative studies.

The Problems of Correlative Studies

There is to be heard on every side a complaint that the various biological disciplines are becoming too highly specialized, that one may be a physiologist, biophysicist, biochemist, anatomist, morphologist, ecologist, mycologist, algologist, pathologist, bacteriologist, systematist,

geneticist, cytologist or any one of other innumerable "ists," but no longer a botanist or zoologist—and certainly never a biologist. Yet those who raise their voices in lamentation might remember that there was a time when to be a biologist was also to be what we call a taxonomist (or more recently a systematist) for that was the only kind which then existed.

In viewing the fields which with bewildering multiplicity have grown up under the general heading of biology, there may be those who sigh for some one to see the Burning Bush—a Moses who will lead us through the wilderness and into a promised land. Yet I have sometimes wondered whether this so-called specialization is as real as some would lead us to believe.

The physiologist, if he is to keep abreast of his science must, among various other things, also be a chemist and physicist. In like manner, the ecologist must not only be well grounded in physiology, but must also be a biogeographer (which also entails a knowledge of geo-history) and, if a plant ecologist, he must know something of the intricacies of soil science—he must also be a pedologist. And it is quite likely that the developmental morphologist is to-day equally trained in anatomy, cytology and physiology, as well as their necessary ramifications which partake of yet other fields. In considering the growth of biology as an organized science developing out of systematics I can not take the view that any of its recognized segments are overly specialized, for they consist of an interlocked system of disciplines, each with its own techniques and methods of approach. If there is specialization to-day it is that imposed by the limits of the individual's ability to encompass or make use of only a portion of man's accumulated knowledge during his normal life-span. No segment of biology—or, for that matter, any science—can exist as an entity apart from others. Yet, throughout the later development of biology, systematics remained apart (or thought it did), for there were

many who held that "the taxonomist needs only to know taxonomy."[2]

There is, however, a constantly increasing group of workers not completely satisfied with the orthodox techniques of systematics. It is this group of workers which is giving impetus to biosystematy. But the student of this broader phase of our science, to do effective work, needs to be conversant with more than the current issue of the International Rules of Nomenclature. It will not be required here to outline in great detail the necessary training of a biosystematist. Briefly, in addition to a familiarity with the usual techniques of the systematist, he should have basic training in such disciplines as morphology, comparative anatomy, cytology, genetics, physiology, ecology, biogeography, physiography and historical geology. And by "basic training" I do not mean introductory or "survey" courses; I mean training sufficiently advanced that he can not only read and interpret the general and technical writings of these fields but also apply their findings to the solution of his own problems.

The time is approaching when the systematist will no longer be completely free to sit before a pile of museum specimens and whittle out species as he may choose, confident that his results will be accepted as authoritative. It will be necessary that they have physiological and ecological soundness, that they fit into their physiographical niche, and that they do not fundamentally conflict with functional biogeography. Also, the historical geologist has every right to inquire into the soundness of the systematist's interpretation of the distribution and phylogeny of a group, for phylogeny, distribution and time are closely linked. Also, the cytologist, peering down the barrel of his microscope, can often see things which confound the careless systematist, while at the same time the geneticist can be more than embarrassing with his ques-

[2] It is true, the taxonomist (or systematist) did make use of morphology, but only such of its findings as would fit into his tidy filing system. Even to-day, every systematist must deal with forms which are morphologically "anomalous" in the group where they are placed.

tions. And since morphology and comparative anatomy both deal with the structure of organisms, the queries of specialists in these fields must be given consideration. Since they all are users of the names of organisms they have every right to expect that these names not only be attached to population units which are biologically sound, but that these names represent concepts of units which are both useful and usable.

He would be a paragon of perfection who would be a well-trained systematist and an expert in all these other sciences at the same time. That is perhaps too much to ask. Yet, if he is to be effective, the systematist must be able to look at his work with something of the perspective of workers in all these fields combined. Furthermore, it is the systematist's privilege also to ask that the workers in these other sciences understand something of his problems: that they also have some training in practical systematics. Greater cooperation between the various fields of scientific endeavor—based on an understanding of the techniques of each—would certainly yield results that would be mutually helpful.

The ways in which the various branches of science can cooperate are innumerable and it would be folly for me to attempt to list them here, even those which have a direct bearing on systematics. However, as a point of departure for possible future discussions, some of the ways will be briefly considered in which systematics, especially of plants, may be more closely linked with other branches of science, and to their mutual benefit.

Historical Geology and Paleogeography: There would seem to be a great gulf lying between the activities of the systematist attempting to apply names to the present floras and faunas of the world and those of the geologist whose concern is an understanding of past continental forms, as well as the construction of an adequately correlated time-scale which reaches back untold millions of years. Yet these fields are not so remote as we might suppose, for wherever possible the geologist relies on the

remains of fossilized organisms for his correlations. The paleontologist, therefore, is a systematist of the past. Unfortunately, too many paleontologists are orthodox geologists with little or no biological training. They see nothing inconsistent in describing what certainly were tropical, or at least subtropical forests lying above the Arctic Circle. If asked how this could be, how such plants could exist under conditions of extreme cold or long periods of darkness they have replied that "plants were different then" or else have invented such interesting hypotheses as a "wandering pole."[3]

In spite of its acknowledged plasticity and diversity, there is a common pattern to life which dare not be ignored, even by the geologist. If his concepts are not consonant with fundamental biology, then it is possible that he should seek some other explanation than that to which he so precariously clings. By being unwilling to view his problems from the standpoint of fundamental biology, he has closed the door to cooperation with the biosystematist.

In dealing with species the systematist is often plagued with the problem of disjunct distributions. Here is one place where the systematist and historical geologist certainly have a common meeting ground. Confronted with the situation of markedly disjunct distributions of living forms, systematists often sit under their favorite vine or fig and whistle up hypothetical vectors such as wind, water or birds to explain the facts for them. I would not say that these agents have not been active. To do so would be biologically unsound. I would only suggest that distributional disjunction *could* be an indication of former land connection and that a serious review of the problem from this standpoint might yield information pertinent to the solution of the problems of both the systematist and paleogeographer. It is my firm conviction that a time will come when the geologist will look as much to the

[3] Chaney (1940) has pointed out that the wandering pole hypothesis leads to no valid explanation, but only to greater difficulties than before.

modern distribution of floras and faunas for information in the reconstruction of paleogeographic areas and the history of the past (especially that of the Tertiary) as he does to the fossils in the rocks.[4] It is encouraging to note that paleobotanists dealing with the Tertiary are making increased use of the herbarium, seeking for cognate forms among the living species.

Ecology: The field ecologist, dealing as he does with mass populations, may note that what have been called species in current manuals may not react similarly throughout their distributions. Such situations may cause serious difficulty in his interpretations. Rather than discuss the problem at length, I will briefly outline one example.

The problem of the beech (*Fagus*) of eastern North America is well known to ecologists. In a systematic study now in progress this population has been found to be composed of several basic, genetic archetypes, each with fundamentally different ecological requirements. The ecologist, therefore, has not been dealing with a single genetic type which reacted differently in various environments, as he had supposed, but with the reactions of different genetic types resulting in organisms which are differentially selected by the environment. The problem of the ecology of the ''American beech,'' therefore, is also a problem in its systematics. This is only an isolated example of the many which might be cited. The

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[4] For example, Schuchert (1935) attempted to make use of the distribution of modern organisms in his study of the Caribbean-Antillean region. It is to be regretted that no adequate analysis of the vegetable life of the region was available to him or would be even to-day. Excluding the ubiquitous pan-Caribbean strand-forms, the uplands within and immediately surrounding the area contain no less than ten thousand species of important ''index plants'' whose distributions are as yet but poorly known. Some areas, as the important Santa Marta massif lying on its southern border in Colombia, an area rising from tropical jungles to permanently snow-capped peaks—and which probably has been emergent since before the Cretaceous—are as yet botanically almost unexplored. An outline of the problems of trans-Caribbean migration may be found in a recent study by Camp (1941). In a brief but interesting paper Dickerson (1941) made pertinent use of biotic evidence in his study of Pleistocene Malaysian land-forms.

corollary to this is that the ecologist can report the findings of his studies in a precise manner only to the extent that the names he uses are critically applied.

Biochemistry and Physiology: I would first wish to emphasize that there is a response-plasticity within the individual, but it is becoming increasingly apparent that the morphological and physiological diversity within a species—its intraspecific variability—may not so much be the result of responses of its individuals in different environments as the expression of slightly different genetic systems. The basis of genetics lies in the fact that morphological characters are genetically controlled. It would therefore follow that the real genetical control is to be found in the regulation of the physiological reactions which are basic to morphological expression. And since physiological reactions result in the production of various compounds, a biochemical and physiological study of a group of organisms may yield corroborative material quite as valuable to the systematist as a study in morphology.

There has been some consideration of the correlation between biochemical reactions and systematics. In general, however, they have dealt with the broader aspects of phylogeny rather than with the delimitation of species. In a recent paper, Krukoff (1939) remarked: "chemical data seldom have received taxonomic consideration in the past, probably because in many cases, chemical work was done on unauthentic materials." He also notes that "The paralysis potency values and certain other chemical data for seeds of various species [of the alkaloid bearing *Erythrina*] . . . would seem to indicate that the soil and climatological conditions are of much less importance than the inherent characters of the plants. The complex and [morphologically] variable species, as a rule, show considerable variations in chemical values, unlike those species that are uniform in their botanical characters and have a limited [geographic] range." He adds: "I think that eventually chemistry will . . . be of more and more

importance to the taxonomic consideration of plants.''
It is obvious that, for the present, such activities will
center primarily around groups of economic importance,
but other problems involving organisms of no immedi-
ately apparent economic value may also yet be solved by
such correlated researches. An understanding of the
fundamental biology of a group and the refinements of
its nomenclature can go hand in hand.[5]

Morphology and Comparative Anatomy: By the very
nature of his techniques the systematist is something of a
morphologist, for the basis of his methodology lies in a
comparison of differences in structure. Yet there are
many structures hidden beneath the surface of an organ-
ism's epidermis which the systematist of plants has long
ignored. However, there is a growing body of informa-
tion that such characters are of immediate concern to the
worker interested primarily in the delimitation of genera
and species. This, the zoological systematist learned
long ago.

Botanists of all persuasions have been prone to think
that the herbarium is only a storehouse for records of
distribution. It is therefore encouraging to note the
growing tendency of morphologists and comparative an-
atomists to visit the herbarium in order to obtain material
for study. It is admitted that such material is not always
in the best condition. Nevertheless, for certain types of
work it is adequate and serves at least to fill in the gaps
where better preserved material is not available. At the
same time the systematist may realize that the group is in
need of careful revision and welcomes any assistance in
clarifying phyletic lines.

[5] That such studies should sometimes be carried out on a broad front is
indicated by the questions of specific limits in certain populations of *Pyrola*,
where some, which are aphyllous and without chlorophyll, have long been
considered to be different species from those which have chlorophyll-bearing
leaves. Since both types are associated with mycorrhizal fungi and inter-
grades are known to occur, the problem is no simple one for the systematist.
In a recent discussion of the situation in this genus and its problems (Camp,
1940) it was stated that ''the ultimate solution . . . does not lie in her-
barium study but, rather . . . with aid from the geneticist, the physiologist,
and mycologist.''

However, in all too many instances, when the systematist seeks to correlate his revisions with the work of the anatomist and morphologist, to his dismay he finds that these workers have failed to give adequate citation of the material upon which it is based. As a further extension of this principle, anatomists and morphologists should acquire the habit of collecting authenticating herbarium specimens at the same time that they collect their preserved material—and from the same organism. All such specimens should be given adequate citation in the literature along with the morphological discussion. For the anatomist to say that two species—closely related in former taxonomic treatments—have quite different vascular systems is meaningless to the systematist unless backed by authenticating specimens; the systematist is too aware of the pitfall of misidentification.

Again, I emphasize the necessity of workers in one field knowing something of the problems and techniques of those in other and increasingly more closely related fields. If the morphologist and comparative anatomist complain that systematists do not pay sufficient attention to their work, the fault may not lie in the nature or quality of these researches, but only in the manner of their presentation. It may be necessary for the systematist to ignore such work (much as he regrets to do so) because there is no way in which it can be correlated with his own researches by means of authenticating specimens deposited in a herbarium and correctly cited in the literature so that he knows where this material may be found.

Cytology and Genetics: These disciplines probably should be treated under separate headings, but they are so closely linked that for our purposes they perhaps can better be discussed together. As Anderson (1937) has pointed out: "Cytology is unique . . . in that it may indicate not only a difference between species or groups of species but may also demonstrate the way in which these differences came about. It is evidence as to the architecture of the very germplasm itself and is, there-

fore, of more fundamental importance than the mere architecture erected by the germplasm.   Cytological evidence, therefore, can do more than discriminate between species; it can illuminate species.''

When the systematist is confronted with a complex situation it is often imperative that the work of the cytologist be put to practical test by means of the controlled breeding methods of the geneticist, for, on a somewhat smaller scale, he can often reproduce the complexities of a wild population.   At times there may be but little in the findings of the cytologist to tell which segments of a population are intersterile or intefertile, whereas this is one of the first things that the geneticist learns.   Conversely, the geneticist can rarely tell us how species came into being, while this is perhaps the special province of the cytologist, since he is a student of the architecture of germplasms.

For the systematist, cytology is an excellent tool, for, as Anderson also said, the cytologist ''after a few routine examinations, will be able to estimate for the systematist, in advance of the morphological information, the probable evolutionary pattern of the genus or family he is about to monograph.''   And the geneticist can corroborate or disprove his preliminary hypotheses concerning any unusually complex segments of the group as are also found to be present.   Cytology, systematics and genetics—they are an ideal combination when properly correlated, as witnessed by the excellent results of the correlated studies of Babcock and Stebbins (1938) on *Crepis* and those of Clausen, Keck and Hiesey (1940) on a wide variety of genera and species.

The practical aspects of controlled breeding as practiced by the geneticist is at once apparent to the systematist, and there would seem to be no need on my part to elaborate on the extended discussion by Anderson (*loc. cit.*) on the relation of cytology to systematics.   Therefore, I will pass by these points and proceed to several items of less interest, but nevertheless of considerable importance to our topic.

In the development of cytology much stress has been laid on the number of chromosomes found in the individual cell of an organism. I would not minimize the importance of these findings, but the systematist, adventuring for the first time into biosystematy, should not be led astray by mere numbers. They must have cytogenetical interpretation and be derived from a sufficiently large sample of the population before they become significant. To say that a species is characterized by a certain number of chromosomes and this based on the examination of a single individual, or even the individuals of any particular region, may lead to serious errors. Anderson's work on *Tradescantia* (as reported in his 1937 paper cited here, and in others) is proof of the regional nature of cytological variants; for much more complex situations, the papers by Wahl (1940) on *Carex,* and Winge (1940) on *Erophila (Draba)* may be consulted.

Yet there is a value, even to the studies of individual organisms, if they are done in such manner that they become common scientific property. It may appear that I am scolding, but this is not my intention. The minor tragedy of so many of these reports is that, from the practical standpoint, they are of no use to the systematist, for they are not backed by authenticating herbarium specimens.[6]

[6] It is true that many chromosome counts are made on root tips, and these often from sprouting seeds which would not be easily identifiable. There are two ways out of the situation. A set of plants could be raised from the same lot of seed; or, better yet, the plant which bore the seed (or at least a suitable portion of it) could have been sacrificed as authenticative material to be cited as has been mentioned in the foregoing paragraphs under the heading of ''Morphology and Comparative Anatomy.'' Where parts other than sprouting seeds are used there is generally ample opportunity to obtain adequate specimens from the same individual, thus insuring against the loss of a single authenticate. Wahl (*loc. cit.*) in his cytological studies of *Carex,* has adopted a method which is to be commended. Three sets of adequate specimens were made from the various individuals studied, two of these being placed on deposit in separate herbaria, and the third designated as the ''traveling set'' to be loaned to any one similarly interested in this complex genus.

In going through the more recent cytological literature, it is encouraging to find an increasing number of students who realize that our current concepts of specific limits are not necessarily valid, that the names we use to-day may not be those we will use in the future, and that a report based on a few individuals may not represent the condition throughout the entire distribution of a species. It is probable that each worker could devise some suitable way in which to authenticate his findings and thus give reasonable assurance of the permanence of the record and also that the material is available to systematists, who will certainly use it in future studies of the group. It is suggested, in addition to the usual data which would appear on the label, that the chromosome count also be appended, as well as a citation of the place of publication containing discussion of the data. In such manner the work of the cytologist, with but little additional effort, is brought into close correlation with that of the systematist. Likewise, the geneticist should also learn that the herbarium is a place where he can store his validating records. And if the specimens are adequate, the systematist will find them of considerable help in his work.

Systematics with Limited Facilities

In viewing the broadening scope of systematics, it is likely that many workers, particularly those in the smaller institutions, may feel that the lack of facilities will seriously hamper their studies. Nevertheless, it is my firm belief that it is just this body of workers, in the aggregate, who will yet make the greatest contribution to the science of systematics. This may be briefly discussed under two headings: biosystematy and mass collections.

Biosystematy: There is no need, even in this expanded phase of our science, for elaborate paraphernalia. An ordinary microscope of good resolving power, a few simple stains, a bit of garden or a small corner of a greenhouse will suffice. Nor is it necessary that we gather material from the farthest corners of the world. The

species at our doorsteps for a long time will yield information as startling and important as those from far-off places. Here is one of the new biological frontiers.

Also, from the academic point of view, an active project in biosystematy under the supervision of even a single instructor would yield so many and such varied problems of immediate and practical importance that it would be a veritable fountain of subsidiary topics which could be parceled out to a series of students working for their higher degrees. Thus, although each student would be primarily specialized in his techniques, he would, by contact with the other phases of the problem, receive a more rounded background in his general training than he might otherwise achieve.

Mass Collections: It is also probable that there may be some workers who do not have available even the small plot of ground necessary for the growing of living plants, or who have no great inclination toward the cytogenetic analyses necessary in biosystematy. They may still feel that work with herbarium specimens is the ultimate in systematics. Yet such a worker may complain that he does not have on file a sufficiently representative number of species of a group to do effective work. To-day, this is no longer an excuse for shirking active systematic research of a most significant type. No herbarium, no matter what its size, can give us the real information we need. It can be gained only by going out into the field and returning with sufficient material, carefully collected, and with adequate data. The herbarium is only the laboratory where the final stages of the research are conducted and where the material used is stored for future reference. It is in these extended studies of large populations—validated by mass collections—where the systematist lays the real groundwork for a study of species.

For example, it may be known that a species has both pubescent and glabrous phases which, in a particular area, may appear together. Since they seem to "intergrade" the systematist may think that the differentiation

of such "forms" is of little importance and that "they are likely to be met with throughout the range of the species." A careful census of a statistically sufficient number of individuals in various areas may indicate that there are no "intergrades" but, rather, that there are different *proportions* of these individuals in different areas. In this manner the student is taking the first and necessary step in the genetic analysis of a species. As Krukoff (*loc. cit.*) pointed out, in *Erythrina,* where important medicinal compounds are sometimes present, minor differences in morphology may be an indication of the presence of exceedingly different biochemical constituents. The extension of this principle is certainly true in the majority of species and reaches practical application in horticulturally and economically important groups. This is the functional phase of systematics. Those who have had anything to do with the selection of material from the wild for breeding-stock in horticultural work know how important apparently minor characters may be as "indicators" of more important items.

There is no opportunity in this place adequately to discuss the value of mass collecting as applied to an analysis of species, or the ways in which it may be carried out. However, for those who are interested, I will refer only to the recent work of Fassett (1941) on the *Rubus odoratus* and *R. parviflorus* complexes. There are those who would seek to solve the problems of this troublesome genus by the description of hosts of utterly useless "new species." By so doing they are bringing us no closer to an understanding of the group; they are only further confounding us. Professor Fassett may lament the fact that his material can not be filed so neatly in an orthodox manner in museum pigeon-holes as the curators of some herbaria might wish. But his species do represent living and dynamic populations. It is this kind of interpretation which we systematists must put on species if our work is to be effective and truly significant.

To many the sole function of the herbarium is that of a place where records of distribution are kept. The collecting of new county or state records, or the local appearance of newly adventive weeds, or their spread, is certainly to be commended, for it is a valuable addition to our knowledge. But such activities can not lead us to an understanding of species. The systematist who neither is concerned with nor makes an attempt to study and understand the nature of the internal variability within species and species-groups is, to-day, scarcely worthy of the name. He has closed his consciousness to a century of research directed toward an understanding of the origin and cause of the diversity of living organisms; he is still traveling under the banner of those who believe in the perpetual fixity of germplasms. His counterpart is to be found in the collector of postage stamps who knows neither geography nor history—and who does not want to learn.

The systematist, being constantly berated by his confrères in other fields (who demand from him peculiarly static concepts which they would not tolerate in their own studies), has attempted to maintain an archaic *status quo*—even by the use of rules. And here, perhaps unknowingly, he has also failed. It would therefore seem only logical that systematists venture forth more often among populations of living organisms, complex and confusing as they sometimes are. With our present knowledge of the causes of this variability, elementary as it certainly is, we may find these populations nomenclaturally less troublesome than we had supposed.

Conclusion

We are now standing on the threshold of a new era in the science of systematics. The day of the taxonomist who putters alone in his herbarium with an other-worldly stare is done. He must shed his robe of academic classicism and seclusion, brush off the accumulated dust of the centuries, and come face to face with the dynamics of

living populations. To-day, as never before, workers in all fields of pure and applied science are beating a path to the herbarium door, seeking for help in the solution of their own problems. There must be no shirking of our responsibilities. We must no longer be mere filers of dead specimens; we must be namers of living organisms. And we must have as an ideal that these names should be applicable not only to population units which are biologically sound, but also that they be of such a nature that they are both usable and useful to all those who have need to know the names of organisms.

It is therefore fitting on this occasion that we dedicate a herbarium to the continued memory of one who was a collector of specimens. (And he was a good one. I know, for I have studied many of his gatherings from northern climes as well as from steamy tropic jungles.) But he is much more than a collector and namer of dried specimens. He is also known for his firm grasp of many of the fundamental problems of biology and his numerous contributions to their better understanding. I am certain, if the choice were his, that this herbarium—built mostly through his own toil—would not be dedicated to himself, but to a continued study of the nature of living things.

Let this spirit of his be with us—always.

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PARASITISM AND EVOLUTION[1]

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THE term "Parasitism and Evolution" has various connotations for biologists. To some, it refers to the modifications that parasitic organisms have undergone in their descent from free-living ancestors; to others, it means the ways by which parasites may have affected, biochemically or otherwise, the evolution of their hosts. In some cases, it is believed that parasites may even have caused the extinction of the forms attacked. Still other workers are impressed by the remarkable series of adaptations which many parasites have undergone for development in and transfer to various hosts. For example, in the life history of a single parasite, there may be involved as many as four separate hosts; these may live in various habitats and belong to widely separated taxonomic groups.

In this discussion, however, another phase of the problem of parasitism and evolution will be considered. This is one in which it seems to me that speculation has often outdistanced the factual evidence. The result is that a generalization has come to be accepted widely although it is based on only a few examples, while the exceptions, I believe, have been all too commonly glossed over.

I am referring to the view that pathogenicity to the host is really a disadvantage to the parasite and that consequently long-standing parasites, by the processes of evolution, have much less of a harmful effect on the host than have recently acquired ones. According to this conception, it is mainly the latter which are productive of severe or even fatal disease.

This view carried to its extreme has led to statements in recent literature such as "a high degree of pathogenicity of a parasite may be considered *prima facie* evidence

of a recent and still imperfect development of the host-parasite relation." (Chandler, 1940.) Or, "a parasite pathogenic to its host is considered to be relatively new to it." (Fantham, 1936.) Or the supposed corollary of this proposition: "There is a sort of mutual adaptation between the two which is developed in proportion to the time that the relationship of host and parasite has existed." (Chandler, 1940.) Various investigators have come to rely upon the type of parasite-host reaction as indicating, therefore, the length of time a parasite and a host have been associated. If the reaction is severe, resulting in disease or death, then the parasite is relatively new for the host; if the relationship results in no reaction or only a slight one, then so runs the argument, host and parasite have been long associated. (Hegner, 1926.)

Let us examine briefly some of the evidence on which these rather sweeping conclusions are based. Perhaps one can illustrate some of the difficulties which follow from the line of reasoning by referring to the amoebae of man's digestive tract. Conservatively speaking, we may hazard a guess that one out of every five persons in this country harbors a harmless or a commensal amoeba in his intestine and one out of every 20 a harmful or pathogenic species. Should we assume that the mere fact of pathogenicity is an indication that the latter species has been more recently associated with man than have the harmless ones in the same environment? On the other hand, all the commensal and pathogenic species of human amoebae are present in the higher apes, and if man, as seems probable, acquired his infection from them, the degree of pathogenicity is hardly a reliable guide to the length of time man and his intestinal amoebae have been living together.

It is difficult to discover who first formulated this idea of an evolution occurring in the degree of host-parasite relationship, with the result of a gradual loss in pathogenicity. Perhaps Van Beneden (1875, '1885) in his "Animal Parasites and Messmates" of nearly 70 years

ago was one of the first to set forth the view fairly defi-
nitely when he wrote: "The Parasite is he whose profes-
sion it is to live at the expense of his neighbor, and whose
only employment consists in taking advantage of him,
but prudently, so as not to endanger his life. He is a
pauper who needs help, lest he should die on the public
highway, but who practices the precept—not to kill the
fowl in order to get the eggs. It is at once seen that he
is essentially different from the mess-mate who is simply
a companion at the table. The beast of prey kills its
victim in order to feed upon his flesh, the parasite does
not kill; on the contrary, he profits by all the advantages
enjoyed by the host on whom he thrusts his presence."
(Beneden, 1885, p. 85.)

There has been a gradual development of this concept
since it was first proposed, and such eminent authorities
as Woodcock in Lankester's "Treatise on Zoology"
(1909), Minchin (1912) and Theobald Smith (1921) did
much to popularize it and to apply it to modern con-
cepts of epidemiology. There is indeed strong circum-
stantial evidence that such adaptations, resulting in
decreased pathogenicity with long-continued parasitism
have occurred many times in the past and are perhaps
still occuring to-day. But that such occurrences are uni-
versal, so that we may therefore argue back from the
degree of pathogenicity of a parasite to the length of
time the parasite has been living in a particular kind of
host, is a view which, I maintain, is hardly warranted by
the evidence at our disposal.

At the outset there seems to be a contradiction between
two supposed evolutionary developments. On the one
hand, we have the commonly accepted view of the relation
of the parasitic way of life to those types of association
which we call commensalism and symbiosis. On the other
are these views I have mentioned, of decreased pathoge-
nicity with increased length of parasitism.

Let us examine the dilemma a little more closely. Most
parasitologists would agree that free-living forms were

the predecessors of their parasitic relatives and that parasitism in most cases followed upon some commensal or symbiotic relationship. In most instances, those forms which are found in such relationships are more like their free-living relatives than are the true parasites. If this be the case, then parasitism with accompanying pathogenicity is the result of a long rather than of a short period of association, and we are justified in concluding that the pathogenic *Endamoeba histolytica* is really an older inhabitant of man, in terms of evolution, than is the commensal and harmless *Endamoeba coli,* which has not yet learned, evolutionarily speaking, to swallow *in vivo* epithelial cells or blood corpuscles, in preference to the bacteria or the food particles in the gut lumen.

In his paper on ''The Evolution of Parasitism among the Protozoa'' (Fantham, 1936) the late Professor Fantham postulated a phylogeny of the amoebae of man. He believed that the common fresh-water amoeba, particularly of the *limax* type, came first in the series, then followed a human intestinal amoeba such as *Endolimax nana,* in morphology very much like a free-living limax type, next came *Endamoeba coli,* and finally *Endamoeba histolytica,* which, he said, ''has progressed still further along the line of parasitism'' (p. 317).

In the same article, however, Professor Fantham concluded: ''In parasites long resident in their hosts, a mutual state of harmony or tolerance is set up to a greater or less degree. The newer a parasite is to its host, the less is the harmony that exists between them'' (p. 324).

There seem to be two ways out of this dilemma. One is to assume that the non-pathogenic effect of a parasite on its host may occur in two separate periods of the evolution of the former: once, before the parasite has evolved completely from a symbiotic or a commensal state and before it has successfully adapted itself to living at the expense of other organisms; and secondly, after a comparatively long period of parasitism, when mutual adaptations of host and parasite have reduced it to a stage of

comparative harmlessness. Such a thesis can be maintained logically. But in the present stage of our knowledge of the evolutionary past of parasites, there are few parasitologists who would hazard an opinion that parasite A is relatively harmless because, from the standpoint of evolution, it is just embarking on its nefarious career and hasn't learned perhaps how to be successful burglar or pickpocket; and that parasite B is, on the other hand, relatively harmless, because it is an old hand at the game and has learned that if it doesn't steal too much, the loss will hardly be noticed, while grand larceny or murder may quickly cause the destruction of the malefactor. In any case, one can not maintain that relative harmlessness is *prima facie* evidence for long parasitism, and severe pathogenicity equal evidence for its relative newness.

There is, however, another way out of the difficulty, which, nevertheless, seems to me to be even less satisfactory. Rabaud (1928) and others question the usually accepted line of evolution leading from commensalism or symbiosis to parasitism and maintain that each of these relationships came into existence more or less independently (pp. 76, 43–44). Under certain conditions, a host-parasite relationship results in commensalism; under others, it may result in parasitism. According to this view there is no progressive adaptation leading from one stage to the other; commensalism or symbiosis are not stages ending in parasitism; each relationship has arisen all at once and each represents an end-point in any particular evolutionary development.

Such a theory is so much at variance with what seems to be an overwhelming mass of evidence (Wenrich, 1935) for the development of parasites from free-living ancestors through a series of commensal and symbiotic relationships, that few workers would adopt it without more facts than have been so far marshalled in its favor. Furthermore, just as gradual adaptations, by this theory, have not produced parasites from free-living forms, neither have they resulted in a gradual decrease in patho-

genicity with increased length of parasitism. Just as parasitism would have occurred in one jump, so pathogenicity or its absence would occur just as fortuitously and just as independently of the time element.

There are, or so it seems to me, numerous instances of comparatively new infections which have been established in a host with little or no pathogenicity; just as some long-standing parasitic infections may still produce some very serious symptoms. Much of this evidence is to be found in the field of experimental parasitology and some from the known history of disease caused by animal parasites.

The examples chosen have been drawn mainly but not entirely from the group of the parasitic Protozoa. They should be of interest, however, to all naturalists and not merely to the technical parasitologist.

The intestinal amoebae of man have already been mentioned. Dobell (1931, 1933, 1936) in a series of very painstaking researches has demonstrated that all the major amoebae of man are to be found in monkeys. It is theoretically possible that the various primates have acquired their intestinal protozoa from man rather than *vice versa*. But the very wide-spread occurrence of these parasites in certain kinds of monkeys, together with the findings that apparently every major intestinal protozoan of man—amoebae, flagellates and ciliates—occurs in wild-caught monkeys (Hegner and Chu, 1930, 1930a) leads to the view of the monkey-man rather than to the man-monkey route of parasite acquisition.

If man acquired his intestinal protozoa fully formed from his ancestors, they must be fairly "old parasites" from the standpoint of human evolution. In the monkey, as in man, the amoebae are apparently harmless except for one: *Endamoeba histolytica* (Johnson, 1941; Ratcliffe, 1931; Hegner, Johnson and Stabler, 1932; Eichhorn and Gallagher, 1916), which may be highly pathogenic or even fatal. Are we justified, therefore, in singling out the pathogenicity of this one species of intestinal amoeba as

"*prima facie* evidence" that it is a recently acquired parasite, and in concluding that the harmlessness of the other amoebae of man is in itself an indication of a much longer time that "the relationship of host and parasite has existed"? Such conclusions seem as unjustified for the human amoebae as they are for certain other groups of parasites.

The intestine of man and of other vertebrates harbors not only amoebae but also various species of flagellates. Some of these are highly pathogenic for their hosts; others are apparently entirely harmless. A great many of them may be transferred to and may establish themselves in foreign or what some investigators are inclined to call "abnormal" hosts. Such hosts may be as widely separate as birds and mammals. Here it seems we should find ample evidence for the thesis that high pathogenicity indicates recent parasitism, and low pathogenicity a mutual adaptation resulting from long-standing association.

Two common intestinal flagellates of man and of certain other mammals belong to the genera *Chilomastix* and *Trichomonas*. In most of these hosts, they are non-pathogenic, as a result, shall we say, of long-continued association with their hosts. Young chicks, however, are very easily infected with mammalian species of these flagellates, which carry on their life histories normally in their new hosts. *Chilomastix*, from guinea pigs, as both Hegner (1929, 1929a) and the writer (Ball, 1931, 1932) have shown, will encyst, divide and excyst in the chick cecum; *Trichomonas parva* (Hegner, 1929a, pp. 35-36) of the rat has persisted in fowls for at least 191 days and *T. hominis* from man for at least 141 days (Hegner, 1929).

Here are instances, and only a few are mentioned, for many foreign species of flagellates were found to live normally in young chicks, in which a new parasite is established in a host, but where no pathogenicity results. If such associations should occur in nature, the parasitologist or the evolutionist would indeed be in error in con-

cluding that the non-pathogenicity was the result of a long-standing connection between host and parasite. Again, the effects of the parasite are poor guides for determining the length of association.

But there are other trichomonads that may be highly pathogenic to their hosts. Such a species is *Trichomonas gallinae*. In pigeons, doves and certain other birds, it may produce severe symptoms and even death (Cauthen, 1936; Levine, Boley and Hester, 1941). It is possible to transfer this parasite experimentally to various raptorial birds (Stabler, 1941). Where the infection is successful, disease conditions may develop, resulting in the death of the host; or else the infection may be successfully established, and the birds remain perfectly healthy. Once again, the degree of pathogenicity is the result of various diverse factors and is in no way conditioned by the length of time that host and parasite have been associated.

If we turn our attention to some of the blood-inhabiting protozoa, we find that there are many instances where pathogenicity is unrelated to length of host-parasite relationship. Among the species of bird malaria found in nature, some ten species can be transmitted experimentally to an unfamiliar or, in the terminology of some workers, to an "abnormal" host. (Hewitt, 1940.) Some species are highly pathogenic to the canary, resulting in the death of many birds; while other species produce little or no effect on the experimental host although infection becomes established. If one examines a table (Hewitt, 1940—table 8) of these various species of malaria in a single experimental host, he is struck by the great diversity of behavior. Some parasites of a relatively high pathogenicity develop rapidly, some slowly; some are demonstrable in the blood for a long period of time; some for only a few days. The conclusion to be drawn from a great number of extensive experiments is that all types of variations occur in the behavior of these parasites when they are introduced into foreign hosts.

Some species are highly lethal; some find conditions so unfavorable that they are barely able to get along with very impaired reproductive powers, and others develop in the new host, sometimes more successfully than in the original one from which they were isolated in the wild, without producing any marked untoward effects in their new home.

In addition to the canary, various other birds, both wild and domesticated, have been used in cross-infection experiments with bird malaria. In many instances, these are distinctly "foreign" hosts, such as the chick or the duck, not normally infected in nature with these species (Wolfson, 1936, 1937, 1937a, 1939, 1940; Hegner and West, 1941, 1941a; Hewitt, 1940; Coatney, 1938; Manwell, 1933). Host-specificity seems to be very loose in many of these forms; for example, a species first isolated from a wood thrush has been transmitted to a canary, to a duck and then to a chick (Hegner and West, 1941a). In most of these "new" or "foreign" hosts, the malaria parasite has a very low pathogenicity or is apparently harmless, even though infection may result in extensive invasion of the red cells and completion of the parasite's life cycle with the production of gametocytes. At least one species, on the other hand, was highly pathogenic to ducks (Wolfson, 1940) and moderately so to young chicks (Coggeshall, 1938).

In one series of experiments (Hegner and West, 1941), a strain isolated from the English sparrow killed 60 per cent. of canaries, that is, it exhibited severe pathogenicity in one kind of new host, but the same strain did not produce symptoms in ducks, another new host of a different sort, although infection lasted in some cases at least two weeks and gametocytes developed. In one new host, infection produced severe disease; in another new host, infection was practically harmless; other factors, and not the newness of the host, determined the pathogenicity of the relationship.

With respect to human malaria, certain statements have found their way into the literature which should also, it seems to me, be re-examined in the light of fuller evidence. It has been maintained that native races living in endemic areas show an inborn resistance to malaria. For example, as the result of many generations of adaptation, the Negro is supposed to exhibit less severe symptoms than does the white man, because of longer association between host and parasite (Culbertson, 1941; Faust, 1931).

More recent evidence, however, has shown that this so-called "racial" immunity to malaria is rather the result of "premunition"—a tolerance established as the result of a latent infection occurring early in life (Smith, 1934, p. 69; Burnet, 1940, pp. 284, 285; Sergent, 1935). As Schüffner, Swellengrebel *et al.* (1932) have shown among the natives of Malaya, the tolerance exhibited towards malaria is acquired at the cost of high morbidity and mortality among the children. Even among the Negroes of our Southern States whose refractoriness to induced malaria is well known (Boyd and Stratman-Thomas, 1933) and is possibly to be explained as an acquired rather than a racial immunity, the death rate from malaria is much higher than among white persons in the same area (Brown, 1940). Although this difference in mortality rate may be due in part to inadequate treatment in the Negro, these figures show that even in the adult, malaria is by no means the relatively harmless disease in the Negro that certain writers have implied to be the case.

Furthermore, among those investigators who maintained that malaria resistance was innate in the Negro, it was recognized that these differences were not the same for all species of malaria. Even though the white race was believed to be more susceptible than the black to tertian malaria, there was evidence that no such racial difference existed for quartan (Culbertson, 1941; Boyd, 1934).

One other example from the malaria parasites indicates the unreliability of following the degree of pathogenicity as a clue to length of time host and parasite have been associated. One species of monkey, *Silenus (Macacus) irus*, has been found infected in nature with three species of malaria, which produce slight or no symptoms (Hegner, Root, Augustine and Huff, 1938; Sinton, 1934) in this host. One of these species of malaria usually is fatal if transferred to a foreign host—another kind of monkey, *Silenus (Macacus) rhesus;* the other species transferred to the same host causes only a mild infection (Knowles and Das Gupta, 1932). To complicate the picture still further, the former species can be transferred to man, another foreign host, in whom it usually sets up a mild infection; with the Negro apparently less susceptible than are white persons (Milam and Coggeshall, 1938).

If we consider briefly some of the other blood or tissue-inhabiting Protozoa, we find similar host-parasite relationships. Among the severe infections produced by parasitic Protozoa in man are those caused by the members of the genus *Leishmania.* Two diseases they produce are kala-azar, with a death-rate in untreated cases of probably at least 75 per cent.; and espundia, found in parts of Central and South America, where the symptoms frequently are extensive destruction of the mucous membranes of mouth, nose and throat, with death a not unequal sequel.

Nevertheless, what evidence we possess indicates that "Leishmaniasis," in the words of Adler, a leading investigator (Adler, 1940), "must be regarded as one of the oldest of human diseases." Espundia, in his opinion, was certainly present in the New World before Columbus, since the disease was pictured unmistakably on Inca drinking vessels (Moodie, 1923); and there is good evidence both from the standpoint of epidemiology and from the differences in their intermediate hosts that kala-azar in South America is at least as old and is not a recent importation. If we accept Adler's view of the antiquity

of *Leishmania* infections in man, we have another instance of the failure of a long-standing infection to identify itself as such to the parasitologist through low pathogenicity and comparatively slight interference with the normal activities of the host. If one should object that the time interval in this instance is too short for evolution to have produced any noticeable effect, it may be pointed out that the period of time is of the same magnitude as that which is supposed to have produced immunity in different races of man to trypanosomiasis, malaria or hookworm.

It is from among the trypanosomes that examples are most frequently cited of recent parasitism being accompanied by high pathogenicity and of long association indicated by comparative harmlessness (Caullery, 1922; Woodcock, 1909; Burnet, 1940; Hegner, 1926; Minchin, 1912; Smith, 1934; Hyman, 1940, p. 111; Fantham, 1936; Taliaferro, 1929). The trypanosomes of the wild game of Africa are comparatively harmless, yet some of them (*T. gambiense* and *T. brucei*) may be highly pathogenic when transferred to foreign hosts, such as man or his domestic animals. In fact, some investigators believe that one of the most virulent trypanosomes of man, *T. rhodesiense,* first discovered in 1909, probably came into existence about that time either as a new species or as a race of *T. brucei* capable of infecting man.

No one would be justified in denying that adaptations of this sort have occurred in the history of host-parasite relationships, and evidence for their occurrence is probably strongest in the *Trypanosomidae.* The thesis that is being questioned is rather the validity of assuming that this is a universal phenomenon, so that one can postulate the length of the relationship merely by the degree of pathogenicity.

Even among the trypanosomes, recent observations cast a certain shadow of doubt on these interpretations. Recent investigations indicate that although the wild game of Africa can be infected experimentally with *T.*

gambiense, it is very probable that this parasite does not occur in nature in any wild game animal (Chandler, 1940; Craig and Faust, 1940; Duke, 1936, 1937, 1937a); consequently, we are hardly in a position to determine the ''natural host'' of this particular parasite. It may have arisen as suddenly as did apparently *T. rhodesiense.* On the other hand, the domestic animals introduced by man, such as sheep, cattle, goats and pigs, have been found to harbor the parasite and to serve as true animal reservoirs without displaying symptoms (Craig and Faust, 1940; van Hoof, Henrard and Peel, 1937, 1937a). Both man and the domestic animals accompanying him may perhaps be considered ''foreign'' hosts of *T. gambiense.* The intimate nature of their relationship must have carried the infection from one type of host to the other in a very short space of time. Nevertheless, of two possibly ''new'' hosts for a single kind of parasite—both infected for about the same length of time—one exhibits severe symptoms and one practically none, yet in both the parasite has established itself and become infective for the intermediate host. Lack of pathogenicity in domestic animals is not due to inability of the parasite to complete its life history.

Comparing the situation in *T. gambiense* with that in *T. rhodesiense,* it appears that it is hardly valid to generalize from what seems to have happened with the latter parasite in the face of what seems to have occurred with the former. *T. gambiense* is pathogenic for one supposedly new host, man; harmless in others, certain domestic animals; *T. rhodesiense,* or the closely related *T. brucei,* is pathogenic to both man and domestic animals. That the latter is necessarily pathogenic because it is a new parasite or a new parasite because it is pathogenic (Fantham, 1936), are not conclusions warranted, I believe, when other species of trypanosomes are considered.

In another trypanosome of man, *T. cruzi,* the widespread occurrence of the infection in native mammals in endemic areas and the infection of the intermediate host

in the absence of demonstrable human infection have led
most workers to consider lower animals as the natural
hosts of the parasite (Galli-Valerio, 1920; Chandler,
1940; Culbertson, 1941).   If man is a relatively new or a
foreign host for the trypanosome, he exhibits a low rather
than a high susceptibility, for he is infected with difficulty,
and probably the majority of infected humans show few
or no symptoms.

These are only a few out of a large number of examples
of protozoan parasitism, examples which, I believe, fail
to substantiate the doctrine that severe pathogenicity is
the mark of a new parasite and relative innocuousness the
sign of a long-established one.   There are those instances
where the death of the host may occur as the result of the
infection but subsequent to the completion of the para-
site's life history.   The ciliate, *Ichthyophthirius*, an ecto-
parasite of fish, furnishes an example.   Here the death
of the host is a matter of indifference as far as the para-
site is concerned.   Furthermore, there are to be consid-
ered those relationships where the death and decay of the
host is actually an aid to the dissemination of the parasite,
as in some of the tissue-dwelling Myxosporidia which
produce considerable tissue destruction and often death
of the fish host (Kudo, 1934).   In these and in other cases
of parasitism in nature, long association of host and para-
site has not resulted in a relatively harmless relationship.

Evidence of a similar sort can also be found among the
Metazoan parasites.   Among the more severe worm in-
fections of man are those caused by the blood flukes be-
longing to the genus *Schistosoma*.   In some endemic
areas, they cause "more sickness and death than any
other parasitic disease" (Chandler, 1940); a death rate
of 10–20 per cent. is not at all uncommon among untreated
cases, with severe and disabling symptoms among many
who do not succumb.   In some areas, the infection may
involve 60–85 per cent. of the population.   Here is a para-
site which is highly pathogenic for man, yet it is one of the
oldest human parasites of which we have any record.   Not

only are the symptoms described unmistakably in the papyri of ancient Egypt, but the ova of one species, *S. haematobium,* have been discovered in the bodies of mummies of 1250 B.C. (Moodie, 1923), and there is evidence that the oriental form *S. japonicum* is probably as old (Hegner, Root, Augustine and Huff, 1938). One would not be justified, therefore, in assuming that the severity of *Schistosomiasis* indicates a fairly recent human disease, since we have actual evidence that it is a very ancient one.

The results of experimental work with various helminths indicate that many factors beyond mere length of association play an important part in the incidence and pathogenicity of worm infections in new hosts. Investigators working with hookworms, or *Ascaris,* or tapeworms have shown again and again the important roles played by age, genetic constitution, vitamin deficiency, previous type of host, etc., in establishing infection and in the effects produced on foreign hosts. Rarely does a helminth introduced experimentally into a foreign host run riot and produce severe symptoms. On the contrary, the new host at first is apt to be much more resistant or to exhibit very little effect (Kotlán, 1934; Cameron, 1934; Culbertson, 1941; Scott, 1928). One might cite the great difficulty in producing cross infection between man and pig with their respective strains or species of *Ascaris* (Hiraishi, 1928; de Boer, 1935; Clapham, 1934), or the behavior of cat and dog strains of the dog hookworm *Ancylostoma caninum.*

In this species, it has been shown that a strain isolated from the dog is much more infective to other dogs than it is to cats, and that the cat strain infects other cats much more easily than it does dogs. Moreover, in the cat strain, the infectivity can be reversed after one passage through the dog so that it now infects dogs very readily and cats hardly at all. However, after several generations in cats, it apparently regains its infectivity for its original host (Foster and Cort, 1937). The dog strain is apparently more stable and does not show this reversibil-

ity (Scott, 1930a). It is believed to represent perhaps the ancestral strain (Scott, 1930).

The important point for this discussion is that in a single species of parasitic worm, infection may take place in a "foreign" host with no marked change in pathogenicity. In fact, if we accept the view that the dog is the original host (Foster and Cort, 1937; Scott, 1930), then in the more recent host, the cat, infectivity, size, growthrate and egg production is lowered rather than increased. May we say that here we have a case similar to the change believed to have occurred when *Trypanosoma rhodesiense* arose from the closely related *T. brucei*? In the hookworm, however, the race in the new host was less disturbing than in the old; in the trypanosomes, the reverse was the case. Surely any generalization must take into account either possibility.

Similar observations have been reported for the gapeworm of birds, *Syngamus trachea* (Taylor, 1928), in which a strain derived from the starling was less infectious and resulted in a lower death rate for chickens than did a strain derived from other chickens.

In *Trichinella spiralis,* the organism causing trichinosis, Oliver-Gonzalez (1941) found that the virulence of the parasite was decreased by successive passages through rabbits or guinea pigs, but that its virulence was increased by successive passages through rats.

In this survey of instances where degree of pathogenicity would be but a poor guide for determining the length of time host and parasite have been associated, I have neglected almost entirely those cases offering contrary evidence. But what I have been endeavoring to point out is not that such mutual adaptations between host and parasite may not have occurred. It is rather that generalizations have been drawn from certain examples and contrary evidence has been overlooked. In the face of the kind of examples referred to in this discussion, there seems to me to be grave doubt for acceptance of the statement that "a high degree of pathogenicity of a

parasite is *prima facie* evidence of a recent and still imperfect development of the host-parasite relation." Evolution may, in many cases, have brought about a mutual adaptation between host and parasite resulting in relative harmlessness of the relation, but in other instances no such decrease in pathogenicity seems to have occurred; and in still others as the parasite becomes better adapted for life in its host, it has become rather more than less capable of producing disease.

Perhaps, as biologists, we may all agree on one aspect of nature, namely, its exceeding variety. Even a parasite may choose the course of manifest destiny and find aggressiveness more attractive and more valuable than an existence of peace and symbiosis.

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REVIEWS AND COMMENTS

IN this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution.

REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

Evolution The Modern Synthesis. By JULIAN HUXLEY. New York: Harper and Bros., 1943: 1–645. $5.00.

IT would probably be no exaggeration to call this the outstanding evolutionary treatise of the decade, perhaps of the century. The approach is thoroughly scientific; the command of basic information amazing; the synthesis of disciplines masterly. It is meant for advanced students and particularly for biologists. All biologists will profit by reading the book, and many professional workers sorely need to learn the lessons which it presents so clearly and penetratingly. The dedication "to T. H. Morgan: many-sided leader in biology's advance" will be especially appreciated by American zoologists.

Since Julian Huxley is so well able to speak for himself, and has the habit of epigrammatic summarizations, I present his views in his own sentences, selected and rearranged for this purpose, and occasionally slightly altered in a verbal way. Here are some of his more general ideas on evolution:

Evolution may lay claim to be considered the most central and most important of the problems of biology. Darwinism to-day still contains an element of deduction, and is none the worse for that as a scientific study. Evolution must be dealt with under several rather distinct heads: origin of biologically discontinuous groups; origin of adaptations; extinction; origin and maintenance of long-range evolutionary trends. Comparative Evolution is destined to become as important a branch of biology as Comparative Anatomy.

A large number (possibly the majority) of genes exert their effects through the intermediation of a process operating at a definite rate. Studies on relative growth sometimes lay bare the genetic mechanisms underlying

evolution. Geographical divergence is a general evolutionary phenomenon. Evolutionary change is not completely at random. It is an advantage to an organism to have its normal constitution as harmonious as possible.

Adaptation is strongly championed as a factor almost universally of major and indispensable significance in evolution. I have gathered together some of Huxley's key thoughts on this subject:

The Lamarckian interpretation is neither necessary nor tenable. Modern biology repudiates lamarckism. Evolution is a joint product of mutation, recombination and selection.

Adaptation is omnipresent. It has been all-important in evolutionary progress. Every organism cannot be other than a bundle of adaptations. Many of the apparently useless features used in diagnosing species are correlated characters.

Organisms are selected, not on the basis of a conformity to an ideal plan, not in relation to complete functional efficiency, but on the basis of survival. To produce adapted types by chance recombination in the absence of selection would require a total assemblage of organisms that would more than fill the universe, and overrun astronomical time.

Selection-pressure forces life to occupy every geographical area and every ecological niche within each area. Constant selection-pressure will be exerted, causing adaptive trends, once begun, to be specialized toward a limit [explaining ''orthogenesis''].

Release from competition has permitted abnormal variability and multiplicity of forms, not only for species and subspecies but for entire groups [explaining ''explosive evolution'']. Originally adaptative structures or functions degenerate in the absence of further selection-pressure in their favour [explaining ''degenerate evolution''].

The treatment of speciational processes is superlatively fine. Huxley demonstrates a marvelous command of factual data for all main groups of plants and animals. He avoids the restricted view of a specialist in only one group. His more pointed expressions on speciation may be organized thus:

There are a number of quite different *kinds* of animal and plant species, differing in their mode of origin and in their biological characteristics. We can distinguish four main kinds of species, the successional, the geographical, the ecological, and the genetic [long emphasis is given to the role of polyploidy in the speciation of plants]. Species-formation may be continuous and successional; continuous and divergent; abrupt and convergent; or *reticulate.*

Parallelism may lead to forms which are taxonomically indistinguishable, being evolved independently in several areas. Groups may remain perfectly

distinct though morphologically indistinguishable, but subspecies may differ
visibly more than do good species.

Specific systems become mutually inharmonious or even incompatible
through the sheer accumulation of difference. Isolation is *per se* a cause of
differentiation. The smaller the size of a natural population and the more
perfectly it is isolated, the more likely is drift to proceed to its limit [the
Sewall Wright effect, much emphasized].

We must use a combination of several criteria in defining species.
Undoubted species may cross and yield fully fertile hybrids. In most cases
a species can be regarded as a geographically definable group, whose mem-
bers actually interbreed or are potentially capable of interbreeding in
nature, which normally in nature does not interbreed freely or with full
fertility with related groups, and is distinguished from them by constant
morphological differences.

I have read many simpler species concepts, but none
that has appealed to me as a truer rendering of the facts.

While avoiding the idea of Goldschmidt that "micro-
evolution" is something entirely apart from evolution
proper ("macroevolution"), Huxley rightly holds that
"much of the minor systematic diversity to be observed
in nature is irrelevant to the main course of evolution"—
a sort of veneer. Sudden creation of entirely new "reac-
tion systems" do not appeal to Huxley as the mode of
speciation. Rearranging selected sentences we find his
view to be that:

Sudden and unadjusted advance is impossible. Most adaptive specializa-
tion therefore cannot help but be gradual. The mutations that are of value
for evolution will in most cases be of very small extent, of slight effect.
Evolution will in general proceed, not by the selection of single mutations,
but by the selection of mutations in relation to a favourable combination of
existing small gene-differences. Single-gene differences between species
must be enormously numerous. Gone is any notion of species in higher ani-
mals arising by a single mutation, or even by a few steps. Evolution con-
sists in the accumulation and integration of very numerous and mostly small
genetic changes.

Major evolutionary trends, whether classed as special-
ization or as progress, are to Huxley real phenomena,
distinct from (though comprising) the endless array of
intraspecific and interspecific differentiations. He pre-
sents—though briefly and scatteringly—well worded
statements on fundamental principles of phylogeny.
Some of his key thoughts may be brought together:

When a group is considered as a whole, it will be found in the early stages of its history to be radiating in a number of trends. Evolution is essentially divergent. However, in certain long-range evolutionary trends in animals, parallel changes appear to have played a greater part than was formerly supposed [the "horizontal series" of paleontologists are stressed].

Dominant types [groups] are characterized not only by a high degree of complexity but by a capacity for branching out into a multiplicity of forms. In each epoch a minority of stocks gives rise to the majority, in the next phase, while the majority of the rest becomes extinguished or reduced. This radiation seems always to be accompanied by the partial or even total extinction of competing main types.

The further a trend toward specialization has proceeded, the deeper will be the biological groove in which it has thus entrenched itself. Thus specialization, in so far as it is a product of natural selection, automatically protects itself against the likelihood of any change save further change in the same direction. The result is apparent orthogenesis.

Huxley turns away from reading "purpose into evolution, as earlier men projected will and emotion into inorganic phenomena like storm or earthquake." He strikes out for an inductive concept of evolutionary progress and arrives at an interesting philosophy, which, by again selecting and rearranging the author's key pronouncements, may be expressed as follows:

The distinguishing characteristics of dominant groups all fall into one or the other of two types—those making for greater control over the environment, and those making for greater independence of the environment. Thus advance in these respects may provisionally be taken as the criterion of biological progress. Evolutionary progress consists in a raising of the upper level of biological efficiency. Progress is all-round biological improvement; specialization is one-sided biological improvement. Progress is not the same as specialization. Specialization is an improvement in efficiency of adaptation for a particular mode of life: progress is an improvement in efficiency of living in general. Natural selection can account for progress as well as adaptation. There is no more need to postulate an *élan vital* or a guiding purpose to account for evolutionary progress than to account for adaptation, for degeneration or any other form of specialization.

Only along one single line is progress and its future possibility being continued—the line of man. Man is not within the near future destined to break up into separate radiating lines. For the first time in evolution, a new major step in biological progress will produce but a single species. The future of progressive evolution is the future of man. Man, by now become the trustee of evolution, must work and plan if he is to achieve further progress for himself and so for life.

Distribution and Variation of the Hawaiian Tree Snail
Achatinella apexfulva Dixon in the Koolau Range, Oahu.
By D'Alté A. Welch. Smithsonian Miscellaneous Collec-
tions, 103, 1942: 1–236, pls. 1–12, figs. 1–8. $1.00.

Retreading the ground made famous by the speciational
researches of Gulick and of Pilsbry and Cooke, and cover-
ing it much more thoroughly, d'Alté A. Welch has built
up for us, in this work as in a similar, previous treatise,[1]
a noteworthy picture of local differentiation on an amaz-
ingly fine scale. In a single mountain range with maxi-
mum dimensions of about 12 by 35 miles, in which the tree
snails are largely confined to the spurs from the main
mountain ridge, there are recognized in the one species
78 subspecies, many of which are further divided into
numbered races ("varieties"). The species and sub-
species concepts are those of the vertebrate zoologist:
species are set apart on the basis of nonintergradation,
and subspecies are recognized for the local races that are
distinct enough to warrant naming.

This intense speciation is correlated with the highly
localized and apparently rather stabile nature of the
populations. Each local form tends to have some pecu-
liar features of color or form (of the shell), and regional
complexes show certain common features, particularly in
the color of the embryonic whorls. Populations inter-
vening between subspecies often show intermediate or
mixed characteristics. Some features exhibit ecologi-
cally correlated gradients.

In the maze of noncorrelated individual variations,
however, the author had to search long and hard for
characters that follow the conventional association with
locality and with climatic gradients. By far the most
striking peculiarities in shape and color occur within unit
populations and are repeated almost endlessly not only
in forms of adjacent ranges but also in those that are well

[1] Distribution and Variation of Achatinella mustelina Mighels in the
Waianae Mountains, Oahu. Bernice P. Bishop Museum, Bull. 152, 1938:
1–164, pls. 1–13, maps A and 1–16, fig. A. The 4 other species of the
genus as recognized by Welch remain to be treated by him.

separated (in the microgeographic pattern). The shells twist dextrally or sinistrally almost with abandon; adjacent stocks may be predominantly of opposite twist. Plain, banded and striped color patterns occur on both globose and elevated shells, all at single localities. The subspecies to a large degree are characterized by the relative proportions of these individual variations, and are therefore ordinarily identifiable only by series.

Welch's main contribution has been to establish the precise geographical pattern of the local kinds, rather than to make an intensive analysis of the geographical and individual variation. In fact the only character subjected to statistical analysis, save for the relative proportions of color types that are given for a few forms, is the height of the shell (which may not be genetic), and for this character only the range and the means are presented. Two other shell dimensions (width of shell and height of spire) are given only for specimens of median height, or for extreme variations in height. A statistical analysis of measurements of shell height and width, of spire height, and of length and width of aperture, and particularly of ratios between these dimensions, might afford a basis for a more objective treatment of these variable snails. Experimental studies—at least transferences of populations—are also much needed. Such tasks would be enormous, but we hope may be undertaken, at least for a small area, when peace returns to Hawaii.

NOTICES OF NEW BOOKS

Common Edible Mushrooms. By CLYDE M. CHRISTENSEN. Minneapolis: University of Minnesota Press, 1943: i-x, 1-124, col. pls. 1-4, diags. 1-2, figs. 1-62.—Designed to serve the individual who has had no previous experience with fungi, this publication, contrary to the title, contains accounts of both edible and poisonous species. The term "mushroom" is used to embrace all fungi, a usage which many people will be inclined to question. Although descriptive accounts of the various species are included, the illustrations are the most valuable aid to identification. Many

of the halftones are very good. Fig. 49, however, probably represents *Coprinus micaceus* instead of *C. atramentarius*. The four color plates are disappointing because the colors shown for many of the species are not true to life. *Collybia velutipes, C. platyphylla, Hypholoma sublateritium* and *Lepiota Morgani* are outstanding in this respect. *Lactarius cilicioides*, listed as edible, has been treated as poisonous by some specialists, and because of its similarity in appearance to *L. resimus* and certain forms of *L. torminosus*, must be regarded as dangerous. On page 48 an unfortunate misstatement is made, *i.e.*, "... a mushroom that *exudes droplets of a white or colored liquid* is invariably Lactarius." Actually *Mycena haematopus* possesses a dark red juice, and when its gills are broken, droplets are sometimes exuded. Since this is a very common species, every collector is sure to find it sooner or later.—ALEXANDER H. SMITH.

Biology The Science of Life. By MARY STUART MacDOUGAL AND ROBERT HEGNER. New York: McGraw-Hill Book Co., 1943: i-x, 1–963, figs. 1–555.—The authors have handled well the difficult task of illustrating fundamental biological principles with both plant and animal examples. Ample treatment is also given to the development of an appreciation of plant and animal groups by a study of types. Progressive features are the chapters on applied biology: Biology and Human Welfare; Conservation. Many of the illustrations are new and are good except for the sketches used to illustrate the groups of organisms. Inclusion of the derivation of technical words where they occur in the text is commendable, serving to make scientific terms come to life, and the glossary is exhaustive. The length of the book will preclude its use as a text in any but the longer types of courses.—KARL F. LAGLER.

The Wild Turkey in Virginia: Its Status, Life History and Management. By HENRY S. MOSBY AND CHARLES O. HANDLEY. Richmond, Va.: Game Commission of Game and Inland Fisheries, 1943: i-xx, 1–281, frontisp., figs. 1–68. $1.00.—An excellent monograph in the field of applied ecology.

Meeting the Mammals. By VICTOR H. CAHALANE. New York: Macmillan Co., 1943: i-ix, 1–133, 52 figs., 1 map. $1.75.— In this guidebook there are 66 short, intimate, readable, sometimes anthropomorphic biographies of the mammals that might be encountered in our western national parks. It is written for the interested tourist, who will want a copy in his library. Most of the natural history is good. The interesting illustrations by Walter A Weber add much to the usefulness of the volume.— WILLIAM H. BURT.

The Ring-neck Snakes, Genus *Diadophis*. By Frank Nelson Blanchard. Bull. Chicago Acad. Sci., 7, 1942: 1–144, figs. 1–26, maps 1–4. $1.25.—This posthumous work lives up to the Blanchard tradition of impersonal outlook, careful, cautious presentation of data and meticulous, precise style. Much credit must go to Dr. Gloyd, of the Chicago Academy of Sciences, whose deep and intimate understanding of the author is evident in the excellent editing of the paper.

The lack of an introduction is to be doubly regretted since it is quite certain that one had been prepared which is thought to have stated the author's views on speciation in snakes in general.

As has been shown for *Pituophis, Thamnophis* and other snake genera, *Diadophis* differentiated and spread from a southwestern center. The chief similarities evolved in the end forms to the East and to the West are the lack of ventral pigmentation and the reduced number of scale rows. The western derivatives have retained the primitive dentition but have acquired larger bodies while the eastern races have developed more maxillary teeth and shorter bodies.

An unusual feature, or at least an unusual interpretation of evolutionary sequences in herpetology, is found in the author's speculations on the intraspecific relationships of the races of *D. punctatus*. He hypothecated that the Ozarkian upland form is ancestral; that it became reduced in size and number of scales, as it migrated southward into the Mississippi and Gulf lowlands, to form a new race. These features were retained as the lowland race spread and evolved into another lowland race in the southeastern United States. But, when the southeastern reduced form moved northward into the Appalachian upland habitat (similar to the ancestral one) it evolved into another race which was larger and had a greater number of scales, simulating its ancestor of the Ozarks. If this interpretation be correct, certainly the evidence is strong that the changes taking place with the spread of the species were adaptive, as Fitch found in the western garter-snakes, and not orthogenetic.

The author's use of the term hybridization, as contrasted with intergradation, is somewhat confusing. He apparently uses it to indicate interbreeding not only between distinct species but also between any two subspecies, in the same racial group, which do not have the immediate progenitor-descendant relationship. Intergradation is reserved to indicate interbreeding only between direct-line relatives.

Dr. Blanchard had been actively interested in the natural history of the genus for many years as attested to by other publications and the well-chosen, detailed observations under "Habits" and "Habitats" in the present one.—Norman Hartweg.

SHORTER ARTICLES AND DISCUSSION

ON WHEELER'S PAPER CONCERNING EVOLUTION AND THE NEMERTEAN *GORGONORHYNCHUS*

THIS note has been inspired by the detailed, stimulating, extensive paper by Dr. J. F. G. Wheeler, entitled "The Discovery of the Nemertean *Gorgonorhynchus* and its Bearing on Evolutionary Theory."[1] I am not especially interested in the study of worms, and I certainly would not have recognized a *Gorgonorhynchus* before reading Dr. Wheeler's paper. I have, then, no authority to discuss worms. However, I justify this writing on the basis of my being a student of biogeography, evolution and insular biotas.

In 1932, Dr. Wheeler found a nemertean in Bermuda which is most unusual in that it has a proboscis that is many-branched rather than being of the normal simple type found in all previously known nemerteans. He later described it as a new species of *Cerebratulus*. However, a presumably congeneric species, made the type of the new genus *Gorgonorhynchus* (unknown at the time to Dr. Wheeler), had been found in 1930 in Australia and is now known from several Australian localities. Dr. Wheeler notes that the reefs of Bermuda have been scoured by experienced collectors for years and large collections of worms assembled, but that no one had found *Gorgonorhynchus* in Bermuda before he discovered it. He comes to the highly probable but not certain conclusion that the worm has habits which would have led to its discovery had it been there during the surveys made by Verrill, Coe and others (but might it not have been in Bermuda then, at a low stage in a major cycle of abundance?). He says (p. 481) "It should be observed that *this is the only case that has ever been presented of the discovery of a new type in a small and uniquely isolated locality that had been examined many times previously not only by trained observers but by specialists in the group of animals to which the type belongs.*" The discovery of two species of such a unique genus in two widely separated localities at such recent dates has led Wheeler to summarize a large body of data, and he says, "I have ventured to put forward my view because it carries my conception of the gene to a natural conclusion and because it appears to offer an explanation of what to me is an incredible coincidence."

The "incredible coincidence" which has caused Wheeler to

[1] AMERICAN NATURALIST, 76: 766, 470–493, September–October, 1942.

summarize his views regarding evolution is that these remarkable worms have arisen in Bermuda and Australia spontaneously, *"de novo,"* at the same time. Dismissing the possibility of a recent introduction, he believes that the worm suddenly appeared in Bermuda because it explosively developed from some other species in Bermuda. He says (p. 480) "It is, of course, conceivable that *Gorgonorhynchus* could have been introduced recently into Bermuda either in the natural course of its own methods of distribution or by artificial and presumably accidental transport, and, that, following introduction, it rapidly and successfully colonized the beaches. What data we have count against a natural introduction, for, though it may be asserted with some truth that the nemertean fauna of the world is relatively little known, such a conspicuous type would almost certainly have been observed." The possibility of its accidental importation by man is, says Wheeler, ". . . not worth consideration." And, further, he says "By eliminating the possibility of recent introduction we face a clear-cut alternative: either *Gorgonorhynchus* was present all the time but remained undiscovered by Verrill, Coe and others, or it has arisen somehow *de novo,* sometime between 1903 and 1932."

After studying the distribution and dispersal of plants and animals for some years, I can not see that the possibility of the recent introduction of *Gorgonorhynchus* in Bermuda has been eliminated. The ways and means of occasional transport of organisms about the world are diverse—and how little we actually know about them! It is common knowledge that various kinds of plants and animals are continually becoming established in strange localities far from their homes. Here in Hawaii, where a keen, active body of naturalists has been working for many years, many hundreds of foreign species have become established and have suddenly appeared in numbers. Every year new ones make their appearance. By what means many of these species got here we do not know, but the fact remains that they did get here and have established themselves, and that some are now common and widespread. Certain of these new-comers have proven to be species new to science, and have been described from Hawaiian specimens, but their places of origin have not yet been discovered. Some of them are utterly different from anything seen in the islands before. Some of them represent orders new to the islands' biota. But that is no wonder, for the world is yet incompletely explored, and the number of kinds of organisms is enormous (there are more than 250,000 described species of

beetles alone), yet that number represents only a part of those that will be described.

About 1900, an immigrant insect new to Hawaii made its appearance, and a short time later it threatened to wipe out the sugar-cane industry throughout the islands. The species was found to be new, and it was so distinct that a new genus had to be erected for it. Its native home was not known. At a later date this insect (now known as the sugar-cane leaf-hopper, *Perkinsiella saccharicida* Kirkaldy) was found to be a native of Australia. However, the environmental pressure of its home country is such that the species is not common and is not considered a pest there. Other species of the same genus have since been described from a number of localities. Parasites and predators have been imported to Hawaii and these have exercised such a successful control upon the insect that it is no longer a serious pest in the islands. The annals of entomology record this history as one of the classic examples of biological control. *Gorgonorhynchus* appears to be one of the common nemerteans of Bermuda. Is it not possible that its abundance has the same basis as that of *Perkinsiella?*

The distribution of *Teredo fulleri* Clapp, 1924, described from the West Indies, is interesting and analogous. Recently Dr. C. H. Edmondson has found that the species had become established in several localities in the Hawaiian Islands, and in 1941 he determined that it had also become established at Apia, the port of Western Samoa. In so far as is now known, this mollusc appears to be found only in these widely separated localities. Another interesting observation is that it appears to be rare in the West Indies, whereas it was found to be excessively abundant in Samoa. It appears obvious to us that the only plausible explanation of this erratic distribution is that the species has been artificially distributed through the unintentional aid of man. There are other examples of marine organisms that occur in Hawaii but are elsewhere known only from distant localities.

Gorgonorhynchus may well have reached Bermuda on ship bottoms. Chilton[2] assembled data regarding the dispersal of marine Crustacea by ships. A specimen of the North American horseshoe crab (*Limulus*) was found at Copenhagen, and an example of a Malaysian species was caught at Auckland, New Zealand. Presumably both individuals had been carried to these distant localities by ships. Any one who has seen the diversity and quantities of marine organisms on the bottoms of dry-docked

[2] *Trans. New Zealand Institute*, 43: 131–133, 1911.

ships, especially those heavy with "weed," could hardly doubt that such vehicles must act as effective agents of dispersal.

It appears most probable that *Gorgonorhynchus* has been accidentally imported to Bermuda in recent years. Need it have been imported all the way from Australia? Perhaps the genus has a wider distribution than is now known, and, if so, there may be localities nearer to Bermuda where it will be found. Attention should also be given to the possibility that both the Australian and Bermuda species have been introduced from some region where they are, perhaps, rare and where they have not yet been found. The nemerteans have received little critical study in the tropical Pacific as a whole. The routes of ships, large and small, are often quite distinct from the regular steamer lanes marked on the maps. Yachts, warships and other craft put into island ports from all corners of the world, and some of these might bring in odd creatures from strange lands. Only further collecting will elucidate such problems of distribution, and I believe that it is safe to assume that the species of *Gorgonorhynchus* now known only from Bermuda will be found elsewhere. The erection of evolutionary theory upon such incomplete information is not justified.

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A FACTORIAL EXPERIMENT ON THE MINERAL REQUIREMENTS OF A *DROSOPHILA* CULTURE

Introduction

Drosophila melanogaster has been bred on a diet containing K_2HPO_4 and $MgSO_4$ as the sole salts (Loeb, 1915), NaCl and $CaCl_2$ being present only as impurities. Both the potassium and the phosphate were essential and no flies could be raised if sodium was substituted for potassium; a fly occasionally developed on potassium phosphate alone. In reporting these results Wigglesworth (1939) remarks that "it is unknown what the larvae contrive to pick up from the impurities, for no analysis of the larva ash was made." It is obvious that in Loeb's experiments nitrogen must have been present in organic form, probably in the yeast on which *Drosophila* usually feeds. Making use of these and similar results Pearl (1928) and his collaborators developed an elaborate synthetic medium for a yeast culture of *Drosophila* containing in addition to agar (2 per cent.), cane sugar (8 per cent.), and tartaric acid (0.5 per cent.), five salts in the following concentrations:

Ammonium sulfate $(NH_4)_2(SO_4)$ .... 0.2   per cent.
Epsom salt $MgSO_4, 7H_2O$ .... 0.05 "   "
Calcium chloride $CaCl_2$ .... 0.025 "   "
Primary potassium phosphate KH_2PO_4 .... 0.1   "   "
Rochelle salt $KNaC_4H_4O_6, 4H_2O$ .... 0.8   "   "

As Pearl's medium is still widely used, it is as well to investigate whether the five salts mentioned are necessary in *Drosophila* cultures and whether some may not even be harmful. In addition, it is of interest to determine whether they are given at the optimum concentration. Factorial experiments, so successful during the past few years in agriculture (Yates, 1937), may be expected to give an answer to these questions in a much shorter time than was possible at the time of Pearl's experiments. The determination of the mineral requirements for the usual two species system (yeast-*Drosophila*) may be regarded as a first step towards determining the minimum requirements of *Drosophila* under sterile conditions.

MATERIAL AND METHOD

A Swedish wild-type stock was used in all experiments. Two males and three females, all three to four days old, were kept for a week on the media and then released. The temperature was 27° C, the relative humidity 70 per cent. All the offspring were counted.

In the first factorial series 1,570 flies were bred in 192 vials, each containing 10 cc of 2 per cent. agar solution with 8 per cent. sucrose, 5 per cent. tartaric acid and some cellucotton (cellulose) to give the mixture a higher consistency. Following Pearl's prescription .008 gm Rochelle salts (R), .002 gm ammonium sulfate (A), 0.0025 gm calcium chloride (C) and 0.005 gm Epsom salts (E) were added to the solution singly and in all possible combinations. Potassium phosphate was used at three levels, 0.00 gm, 0.005 gm (P_1) and 0.015 (P_2), Pearl's value being between these last two (0.01 gm).

Sixteen combinations were tested in the absence of phosphate and at the two phosphate concentrations mentioned above. Their constitution was as follows: (1), R, A, RA, C, RC, AC, RAC, E, ER, EA, ERA, EC, ERC, EAC, ERAC.

Of these 48 experiments four replicates were made, giving a total of 192. After cooling the vials were sprayed with a very dilute emulsion of yeast in water.

RESULTS

Of the results obtained only a few need rigorous treatment by analysis of variance, an example of which will be given later. The most important ones can be summarized as follows:

(1) Influence of KH_2PO_4:

In 64 vials containing no potassium phosphate .... 1 fly hatched
" " " " 0.005 gm " " .... 631 flies "
" " " " 0.015 gm " " .... 938 flies "

From these figures it can easily be seen that potassium phosphate is necessary for the development of *Drosophila* and that an increase from 0.05 per cent. to 0.15 per cent. increases the yield. The one fly hatched in the absence of potassium phosphate developed while potassium was available in the form of Rochelle salt, but phosphate was lacking.

(2) Influence of $(NH_4)_2(SO_4)$:

In 96 vials containing no ammonium sulfate .... 43 flies hatched
" " " " 0.02 gm " " .... 1,527 " "

Thus it is clear that ammonium sulfate greatly favors the development of *Drosophila*.

(3) Influence of $MgSO_4, 7H_2O$:

In 96 vials containing no Epsom salt .... 492 flies hatched
" " " " 0.005 gm " " .... 1,088 " "

The addition of $MgSO_4$ raised the yield in each of the eight cases, where potassium phosphate and ammonium sulfate were present. At the higher phosphate concentration the numbers of flies were for P_2A: 19, 11, 35, 25, and for P_2AE: 54, 67, 57, 39. They show a highly significant difference.

Epsom salt, although not so essential for the breeding of *Drosophila* as potassium phosphate or ammonium sulphate, nevertheless approximately doubles the yield of flies.

(4) Influence of $CaCl_2$:

In 96 vials containing no calcium chloride .... 805 flies hatched
" " " " 0.0025 gm " " .... 765 " "

Calcium chloride seems therefore to have little effect on the yield of flies.

(5) Influence of $KNaC_4H_4O_6, 4H_2O$:

In 96 vials containing no Rochelle salt ....:. 996 flies hatched
" " " " 0.08 gm " " .... 574 " "

Rochelle salt thus decreases the yield.

Table I summarizes the results of all the experiments with K_2HPO_4 and $(NH_4)_2SO_4$, and shows the interaction of C, E and C E with R at both phosphate levels.

TABLE I

		P_1	P_1R	P_2	P_2R
		101	17	90	48
C		68	41	33	76
	E	125	66	217	128
C	E	140	50	213	117

From this factorial experiment it would appear that in Pearl's medium calcium chloride is unnecessary, Rochelle salt detrimental and the other three salts necessary. In addition an increase of the concentration of potassium phosphate increases the yield.

Having eliminated two of the constituents, the next step is to find out whether an increase in concentration of the three remaining salts can increase the yield. For this purpose a three-level experiment was made using potassium phosphate, at 0.2, 0.3 and 0.4 per cent., ammonium sulfate at 0.3, 0.4 and 0.5 per cent. and Epsom salt at 0.08, 0.16 and 0.24 per cent. The total yield in the media was less than those obtained at the highest phosphate level in the first experiment, and there was nowhere a significant increase in yield. It would therefore appear that a further increase of the three salts beyond the maximum used in the first factorial experiment is not profitable. By offering P and K in proportions different from those contained in KH_2PO_4 it might be possible still further to increase the yield; but such an increase would probably not be worth the increased complications of method.

SUMMARY

In this paper a synthetic medium is described for breeding *Drosophila* and yeast, which is simpler in composition than Pearl's, and gives higher yields.

Of the five mineral salts used in Pearl's medium, calcium chloride has been found to be unnecessary, and Rochelle salt detrimental. The yield of flies can be increased by increasing the concentration of KH_2PO_4 from 0.1 per cent. to 0.15 per cent. Pearl's concentrations for ammonium sulfate, 0.2 per cent., and Epsom salt, 0.05 per cent., are very near the optimum.

The composition of the medium is as follows:

H_2O	891	gm
Agar	20	"
Tartaric acid	5	"
Sucrose	80	"
KH_2PO_4	1.5	"
$(NH_4)_2SO_4$	2	"
$MgSO_4, 7H_2O$	0.5	"

H. KALMUS

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STUDIES ON INDUCTION OF MUTATIONS BY CHEMICALS. I. EXPERIMENTS WITH HEAVY WATER (DEUTERIUM OXIDE)

VARIOUS chemicals have been tried with a view to inducing mutations; and in some cases (iodine, copper sulfate), apparently significant increases in mutation rate have been reported. These results have been questioned, however, both because the increase in mutation rate was rather small, and because it has not been proved that the chemical substance used actually penetrated into the nucleus (review in Dobzhansky, 1941, p. 46).

The problem of penetration of chemicals into a living nucleus is one of primary importance in studies of this kind. If the chemical is introduced (by feeding or injection) into the body of a higher organism, it has to penetrate many cellular membranes before it can reach the nuclei of the germ cells to produce germinal mutations. If the Drosophila egg is exposed to chemicals, the cellular membranes of particular cells may not be formed yet if the egg is very young, but still the chemical has to penetrate the vitelline membrane of the egg. In both cases, because of the selective permeability of the living membranes, the probability of penetration of introduced molecules is very low, especially where the vitelline membrane is present.

In the present experiments, heavy water (deuterium oxide) was chosen as the chemical agent, partly because its introduction into a living nucleus involves no more difficulty than the introduction of water, the permeability of cellular membranes and the ingestion in food being similar for water and for heavy water (Krogh, 1938). Consequently, if any organism is raised on food containing a high concentration of heavy water, that organism, including nuclei and genes, will be partially built out of heavy water. This has been shown for a wide variety of organisms,

including yeast (Salzer and Bonhoffer, 1936), algae (Bonhoffer, 1938; Reitz and Bonhoffer, 1935), fishes (Hevesy, 1935) and mice (Rittenberg and Schoenheimer, 1937; Stekol and Hamill, 1937; Smith, Trace and Barbour, 1936). The most efficient way of introducing deuterium into Drosophila is to raise the larvae and flies on food containing deuterium. Another way is to inject heavy water into the body cavity of the larva, in which case it may be expected that the deuterium will penetrate the gonads and affect the germ cells.

As a rule, if water containing deuterium reaches an organic compound, hydrogens that are connected with N and O exchange with the deuterium almost instantly (Reitz, 1938; Bonhoffer, 1938), in a ratio which tends to correspond to the deuterium/hydrogen ratio in the surrounding water (Wirtz, 1937; Ogawa, 1936). This exchange is easily reversible, and if pure water subsequently reaches this compound all the deuterium is replaced again by hydrogen. Hydrogen connected with carbon, on the other hand, is not so readily interchangeable with deuterium (Reitz, 1938; Bonhoffer, 1938); the exchange may take days, and at all times the concentration of deuterium reached in the organic compound is lower than in the water surrounding the compound. In the latter case, moreover, the exchange is not so easily reversible, and deuterium connected with carbon remains in the organism for many days or even weeks (Hevesy, 1935; Smith, Trace and Barbour, 1936) after feeding with the food containing deuterium has been discontinued.

The above-described substitutions have been shown to occur not only in the living organism but also in a variety of organic compounds, including amino acids and proteins (Salzer and Bonhoffer, 1936; Stekol and Hamill, 1937).

In the present study, one generation of *Drosophila melanogaster* was raised on or injected with a solution of heavy water in ordinary water; and deuterium was thus given an opportunity to serve as a substratum for building new genes, as well as to replace hydrogen in the already existing genes. Offspring of the individuals raised on deuterium, and thus presumably containing genes rich in heavy hydrogen, were raised on ordinary food; and consequently the genes of the second-generation flies were allowed to revert to hydrogen structure. It was hoped that, since the properties of deuterium are somewhat different from those of hydrogen, the highly complicated molecule of the

gene might be affected by the change from hydrogen to deuterium and back to hydrogen. It was also hoped that this double exchange of hydrogen-deuterium-hydrogen might not be strictly reversible; in other words, that somewhere within this process a mutation in a gene might be induced.

EXPERIMENTS

In the first series of experiments, wild-type flies of Swedish-B stock were raised on standard banana-agar food containing 40, 55.2 and 60 per cent. of heavy water. Forty per cent. is used here to mean 40 weight units of pure deuterium oxide per 100 units of liquid present in the food. Heavy water was furnished by the Stuart Oxygen Company. Water content of the bananas used (about 74 per cent.) was experimentally determined, and 95 per cent. heavy water was added in the quantity adequate to obtain the desired concentration. In the second series, Oregon-R flies were raised on banana food containing 40 per cent. and 60 per cent. of heavy water. The doses 40 and 55.2 per cent. were sub-sterilizing. In the third series, 29 old larvae (just before pupation) of Oregon-R stock were injected with small quantities of 40 per cent. heavy water, and 24 larvae with small quantities of 95 per cent. heavy water. In the Oregon-R series of experiments, the sperm of the emerging males was tested for sex-linked lethals by means of the standard C1B method on regular food containing no heavy water. The numerical data are given in Table I.

TABLE I

RESULTS OF TESTS FOR X-CHROMOSOME LETHALS OF MALES RAISED ON FOOD CONTAINING DEUTERIUM OR INJECTED WITH DEUTERIUM IN LATE LARVAL STAGE

Treatment	Concentration per cent.	No. of flies hatched or larvae injected	No. of fertile males	No. of sperms tested	No. of lethals
Deuterium in food	40	28	13	1496	1
	40	7	4	344	–
Deuterium injected	40	29	7	187	–
	95	24	2	133	–

RESULTS AND DISCUSSION

Not all the eggs of flies kept on 40 and 55.2 per cent. deuterium food hatched, and only a few larvae pupated and emerged as imagoes. The larvae from cultures kept on 60 per cent. deuterium did not pupate at all. Fifty-eight per cent. of larvae injected with small quantities of 40 per cent. deuterium survived

and developed normally, and so did 12 per cent. of the larvae injected with small quantities of 95 per cent. deuterium.

Various physiological and developmental effects of heavy water on organisms have been reported. In Drosophila, Schmidt-Niel-sen and Schmidt-Nielsen (1936) found that reproduction stops at a concentration of 51.5 per cent.; the simpler organisms, for instance yeast cells, can reproduce at 75 per cent. concentration.

The effects of heavy water on development are, at least in part, caused by the fact that heavy water changes (Bonhoffer, 1938), mostly depresses, the velocity of enzymatic reactions. The sterility observed in the present experiments is probably also due to the upsetting of delicately timed and balanced patterns of enzymatic reactions.

The numbers of sex-linked lethals found in C1B analysis were not higher than the numbers found in untreated Oregon-R flies (Demerec, 1937) ; this suggests that deuterium did not cause any increase in mutation rate.

It has been stated before that organic compounds on deuterium substratum must also contain some deuterium atoms in place of hydrogen. From the analogy with amino acids and proteins it may be estimated that in the present experiments the genes contained up to 30 per cent. of C-connected deuterium and up to 40 per cent. of N- and O-connected deuterium in place of hydrogen; yet this did not permanently affect the structure of the gene.

There are at least two possible explanations of these results. One is that the substitution of deuterium for hydrogen did not change either the structure or the activity of the gene. The other possibility is that the gene structure was affected but was able to return to its original form as soon as the deuterium was again replaced by hydrogen.

These results may be similar to the results on virus reported by Miller and Stanley (1941). These investigators found that even if 83 per cent. of amino groups in the virus molecule are acetylated, this molecule, although so considerably changed, nevertheless reproduces in completely normal form.

SUMMARY

Heavy water (deuterium oxide) was introduced into Drosophila eggs and larvae by feeding and by injections. Owing to the high permeability of the cell membranes, similar to the permeability for pure water, it is very likely that deuterium was utilized in building the gene molecules. Concentrations of 60

per cent. in food prevented the hatching of eggs.   At lower concentrations, given in food and as injections, a few flies were obtained.   These were tested by means of the standard ClB method on regular food.   The test did not show any increase in mutation rate.   It is concluded that the deuterium in the gene molecule did not change its structure or else that this structure was temporarily changed but reverted to the original form when deuterium was eliminated from the organism.

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THE ANALYSIS OF THE RELATIVE GROWTH GRADIENTS AND CHANGING FORM OF GROWING ORGANISMS: ILLUSTRATED BY THE TOBACCO LEAF[*]

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THE size and shape of plants and animals distinguish them from each other, and from these differences derives much of classification, comparative biology and even the recognition of individuals. Some organisms attain the proportions of the adult configuration early, others change slowly from the infantile pattern into the adult form. Claws, antlers, or other parts may grow larger and out of proportion to the rest of the body. Regression in form often denotes the effect of an adverse environment and senility involves change of form as well as function. The analysis of the form changes is essential to an understanding of the growth of organisms. This paper presents a method for this analysis which relates the transformed coördinate method with the allometric expression of growth gradients.

I

Toward the end of the nineteenth century it was discovered that a parabolic curve could represent the growth of organisms and of their component parts. About ten years ago Huxley, Teissier and Needham revived interest in this curve for the analysis of growth. They empha-

[*] Presented at the meeting of the American Statistical Association, New York City, 1941.

sized the unequal growth resulting from gradients; calling it at first heterogonic growth, later allometry (Huxley, Needham and Lerner, 1941). The constant k, of the equation $y = bx^k$, is the ratio of the specific[1] growth rate (dy/ydt) of the part (y) with respect to that (dx/xdt) of the whole organism (x). Regions and individuals have been compared and the index has been used as an aid in unraveling evolutionary history and for the classification of animals. A critical and systematic evaluation of the use of this equation has been made by Kavanagh and Richards (1942).

It was soon observed that if values of the relative growth rate k were obtained for a series of parts arranged in order along the organism, the values usually changed systematically from one end of the series to the other. The rate of change in one series might be different from that in another series at right angles to the first. Centers at which k was a maximum were found. These studies were made by comparing fairly large portions of the organism and proceeding as though the value of k were constant within each such portion. It is reasonable to expect that the value of k will be found to vary continuously from point to point within such portions, and that a more accurate knowledge of the growth will be obtained if the analysis is extended to take account of this continuous variation.

Thompson (1915, 1917) demonstrated that an outline of a part of an organism on a coordinate grid could be deformed or transformed by mathematical methods into the outlines of different but related species. De Conick (1936) used transformation to assist in defining the limits for variation separating one taxonomic species from another. A similar method was used by Richards and Riley (1937) to recover the coordinates of developing forms for comparison with the adult form. Such transformations emphasize the development of form and

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[1] The term ''relative growth'' has been used for the growth rate per unit of quantity (dy/ydt) and for the ratio of such growth of a part with respect to that of the whole organism. To prevent ambiguity we will call the former (change in growth/unit of growth/unit of time) the *specific* growth rate.

are helpful in drawing attention to the relative growth
activity of different regions.

It is obvious that there must be a connection between
these two approaches and that knowledge of the relations
is essential to the analysis of the growth of organisms.

II

Suppose tiny bits of material to be embedded in the
growing substance, particles so small as not to interfere
with the normal growth process, but which do not them-
selves grow. By choosing coordinate axes (which are
considered unchanged throughout the growth) for refer-
ence, we can analyze the changes in the geometrical con-
figuration formed by the positions of these markers as
growth proceeds. Such experiments have been accom-
plished by marking rectangular networks on young
leaves and by noting the shape and size changes of vitally
stained tissues.

Gradients in the relative growth rate imply corre-
sponding gradients in the specific growth rate. Specific[1]
rates simplify the analysis and will be used hereafter.
The mutually perpendicular axes will be called x, y, and
z. The position of a point at a given time can be ex-
pressed by these coordinates and time (t). Such a point
as P_1 in Fig. 1 will move a small distance in some direction
in a short interval of time. Nearby points will also be
moving from growth, but in general with slightly differ-
ent speeds and in slightly different directions. As a
result the distance between two such points as P_1 and P_2
will be continually changing. The absolute rate of in-
crease of length of this segment is $d\Delta s/dt$; the specific
growth rate is $d\Delta s/\Delta s dt$. When Δs becomes infinitesi-
mally small, the specific growth rate approaches a limit
which may properly be called the elemental specific
growth rate. This rate is given by a formula derived in
the appendix. There it is shown that the value of the
rate is in general different in different directions from
P_1, so that a tiny sphere centered at P_1 and growing for

a short time will not remain spherical. It is possible to determine the directions of maximum and minimum specific rates and it turns out that these are always at *right angles to each other*.

For the special case in which the rate is independent of the direction the term *isotropic* may be used. Under isotropic growth a small sphere centered at P and growing for a short time would remain spherical. Under *non-*
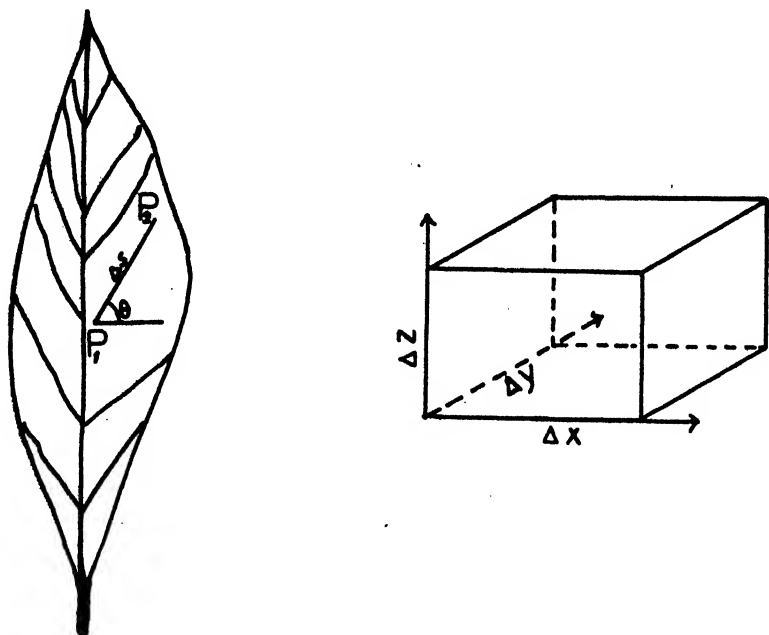
FIG. 1. Illustration of the directions of growth defined in the text.

isotropic growth the sphere would be deformed into some non-spherical shape.

Isotropic growth should not be mistaken for what is sometimes called *isogonic* growth, or change in size but not in shape. Although in isotropic growth a small sphere remains spherical for a short period of growth, neighboring spheres may not grow at the same rate, so that the gross form of the organism is not necessarily maintained. Isogonic growth is a special case of isotropic growth in which the specific growth rate in length

is the *same at every point* throughout the organism, as well as being the *same in any direction* from any given point. The specific growth rate in volume and the specific growth rate in area of a surface can also be expressed in terms of the motions of the points.

III

Few studies of growth are sufficiently complete to provide the basic measurements for a complete growth analysis. Our method is more clearly demonstrated by the analysis of growth restricted to two dimensions than if all three are involved. The best material available to illustrate the method was that on the tobacco leaf of Avery (1933) and we are grateful to Professor Avery for permission to use his material. A young tobacco leaf was marked with a rectangular network on its upper surface. As it grew to maturity the network was deformed by the growth process and Avery's figures 28–31a give four growth stages which will be used to illustrate the analytical method.

The data are from one leaf and we were unable to secure the original measurements, but were forced to rely on enlargements of Avery's published drawings. While recognizing the limitations of the data, we have prepared the present discussion with the idea of illustrating the method and have not hesitated to point to suggested conclusions whose complete verification would require more extensive and more nearly accurate data than were available.

Although growth in the neighborhood of the center of the leaf is continuous, the nature of the markings did not make it possible to use this fact in the analysis. Therefore each half of the leaf was analyzed in turn and the midrib data were used with each half. Slight apparent discontinuities resulting from this fact will be seen.

A description of the procedure used in applying the general formulae to this example is given in the appendix.

The rectangles, marked on the leaf surface, changed shape as the leaf increased in size and attained the adult

form. The corners of the rectangles are the reference
points used in the analysis. For convenience the lines
originally at right angles to the midrib have been num-
bered from 0 to 12 starting at the base of the leaf.

The distribution of values of the specific growth rate
in area is shown in each of the four stages of Fig. 2 by
solid lines along which the rate is constant, in a manner
similar to the use of isotherms on a weather map to indi-
cate places of equal temperature. The curves marked
"100" are those of approximately the maximum rate at
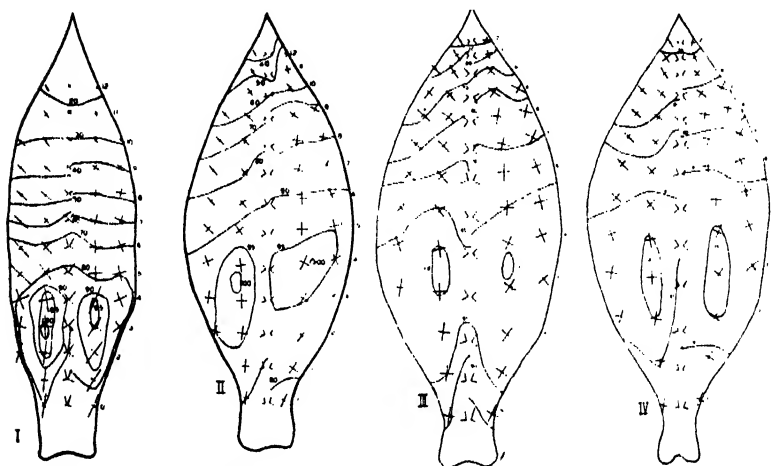
FIG. 2.   Growth of the tobacco leaf; leaves drawn to the same length.

each stage; the numbers "95," "90," etc., indicate that
the rate on the corresponding curve is 95 per cent., 90 per
cent., etc., of that of the curve marked "100."   As a re-
sult of the separate analysis of the two halves of the leaf,
the curve for a given value is sometimes broken at the
midrib; the two parts of each curve have then been joined
by a dotted line.

The tip of the leaf at the first stage, figure 2, is growing
at less than 20 per cent. of the maximum area rate, while
at the fourth stage the tip is growing at a little under 70
per cent. of the maximum area rate. The position of
maximum rate of area growth remains the same near the
lateral marked 3 for all stages. A larger region for

maximum area rate occurred on the left side at stage II
and resulted in asymmetry of the leaf as the smaller com-
pensatory growth at stage III in this region did not re-
move the distortion. As the full size is attained, stage
IV, the regions of maximum rate are nearly equal.

The contours illustrate clearly how small differences
or gradients in the rate of growth can distort the charac-
teristic symmetry, and the regulatory changes that re-
store the symmetry of the leaf. For convenience the
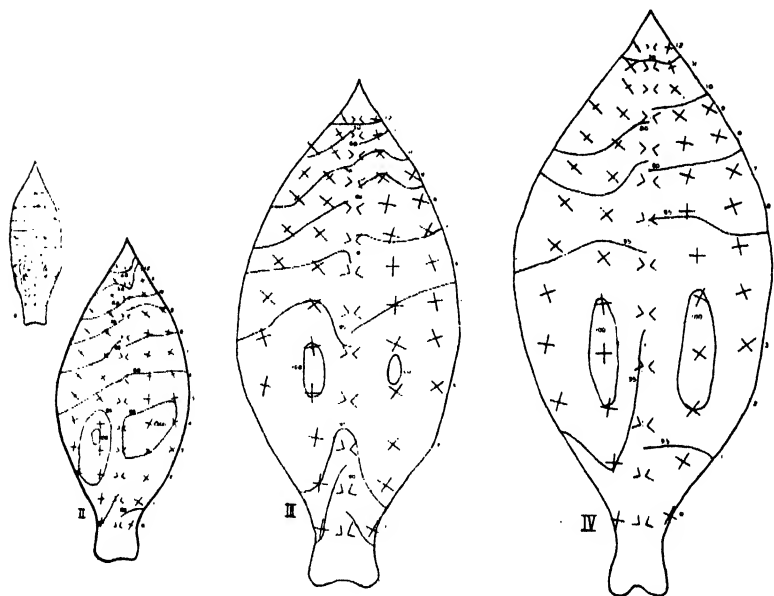
FIG. 3. Growth of the tobacco leaf; size of leaves proportional to their
length at each stage of growth.

stages were made all the same size in Fig. 2. The true
relative size of the leaves is shown in Fig. 3 and the
importance of the changes in growth is more readily
understood even though it is difficult to read the numeri-
cal data.

The nature of the specific growth rate in length is
shown at each point by a pair of crossed lines. The
length of each line is proportional to the specific growth
rate in length in that direction. The directions are re-
spectively those of maximum and of minimum rate at

each point and are at right angles to each other. Due to the separate analysis of the two halves of the leaf, two sets of values were obtained for each point at the midrib; these are shown by half-crosses slightly separated. The specific growth rates for the several points may be compared for the four stages with respect to the relative magnitude and the direction of growth. Note that the maximum rates for the outer points at 2 and 3, especially at stage III, are toward the midrib. This is as it should be; the vascular bundles likewise turn in the same direction, as shown clearly by figure 41 of Avery's paper (1933). The intensity of growth of the leaf axis is depicted graphically; likewise the decreasing gradient toward the tip of the leaf. A striking characteristic of the growth is that the strongly non-isotropic growth of the early stages tends toward isotropism as the leaf grows older. Other interesting and useful comparisons are possible. The active growth regions shown in our figures correspond with the changes in the underlying tissues revealed by the histological studies of Avery (1933).

The ratio of the relative growth rate of a part (growth per unit of growth) to that of the whole would give the quantity (k) of the allometric equation for the relative growth of that particular region to the whole organism. Little would be gained from computing these because different numerical values would be obtained at different stages and from those regions within a stage not growing at the same rate. The k's were computed by Avery for segment area to total leaf area and for length to width of segment; comparing the four stages by pairs. The tabulated values do not reveal the uniformity of the growth as readily as our figures. The more general analysis provides the numerical data to obtain the k's of the allometric method, when these are desired, with the advantage of information regarding the constancy of k (*cf.* Richards, 1935).

Connecting the several points would give the network and would reveal the transformation from stage to stage

due to the growth of the leaf and mathematical expressions for the transformation could be derived should they be required. This aspect is the method of Thompson (1917).

Thus the analytical method derived in the appendix and illustrated above includes both the transformed coordinate and the allometric methods. Other comparisons of the growth are possible. The method may be used for animal growth as well as that of plants and for all three dimensions when adequate measurements of a growing organism become available.

IV

The method becomes somewhat simpler in special cases, such as the growth rotationally symmetrical to its axis. This case has been investigated mathematically, although we have found no suitable data for testing. Certain instructive results may be obtained without the use of data or the presentation of the derivation. Sinnott (1936) concluded for the growth of the pine that, "Evidently the linear relation of pith to the whole could not persist much further without causing a reduction in the absolute size of the tissues outside of the pith." His conclusion was based on measurements of diameters rather than areas. While it is qualitatively true for areas as well, quantitatively the decrease in *area* of the outer bands of tissue will begin much later than the decrease in width . Changes in area rather than in radius may be a better indication of growth.[2] The question of isotropy of symmetrical growth of the type considered in this section shows that a condition for *isotropic growth* is that the growth in any plane section perpendicular to the axis is *isogonic*.

Geometrical change alone, as stated before, may not give a completely satisfactory picture of the underlying

[2] Strictly speaking, Sinnott's study is one of relative size rather than of relative growth, since the data are of Type B of Kavanagh and Richards (1942) classification. Since his successive individuals may be considered to form a series, the same principles hold as in the case of actual relative growth.

growth activity. The change in size at a given point is due to both the functional activity of the cells located there and to the forces of stretch or compression exerted by the adjacent material. The more important these forces, the less satisfactory is pure geometrical change as an index of biological activity. Marked departure calls attention to the need for detailed analysis of the acting forces and thus may serve as a valuable indicator of the order of complexity involved.

The following observations may be made: (1) If the density (mass/unit volume) is increasing in a certain region, the specific growth rate of mass must exceed that of volume. If in addition the volume is increasing, or steady, new material is certainly being deposited. (2) If the density is constant, the specific growth rate in volume just equals that of size. If volume is increasing, new material is being deposited; if it is steady, so is the mass; if volume is decreasing, then material is being removed. (3) If the density is decreasing, the specific growth rate in mass must be exceeded by the specific growth rate of volume. If volume is decreasing, then material is in the process of removal.

CONCLUSIONS

An analytical technic is given relating the transformed coordinate method of Thompson to the Huxley-Teissier-Needham procedure for relative growth gradients and is illustrated with Avery's measurements on the growth of the tobacco leaf. Contour lines were used to show regions of similar specific growth in area and crossed lines on the analytical figures indicate the magnitude of the specific maximum and minimum linear growth, which are at right angles to each other.

Isotropic growth is contrasted with isogonic growth and the special case of growth symmetrical with an axis is discussed. The mathematical derivation of the equations used is given.

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APPENDIX: MATHEMATICAL DERIVATION OF THE EQUATIONS

A. *Basic notation.* If the organism be referred to a set of rectangular coordinates, which conveniently but not necessarily may have its origin at one of the points of the organism, the coordinates (x, y, z) of a moving point may be expressed as a function of time and of their values (x_0, y_0, z_0) at a time chosen as the reference time: $x = x(x_0, y_0, z_0, t)$; $y = y(x_0, y_0, z_0, t)$; $z = z(x_0, y_0, z_0, t)$. That is, each point is earmarked by its position in the reference stage. As growth proceeds, each point moves with a velocity which may be expressed (as in mechanics) in terms of its components $\dot{x}, \dot{y}, \dot{z}$, in the directions of the axes where $\dot{x} = \delta x/\delta t$, $\dot{y} = \delta y/\delta t$, $\dot{z} = \delta z/\delta t$. Then at any instant each of the quantities $\dot{x}, \dot{y}, \dot{z}$, is defined at each point of the organism. Henceforth we shall consider them as functions of the coordinates (x, y, z) at the instant under consideration rather than of the coordinates of the reference stage. Thus, for example, $\delta\dot{x}/\delta x$ means the rate of change of $\dot{x}$ with respect to x, the $y, z,$ and t being held constant.

The *direction cosines* of a line are the cosines of the angles the line makes with the $x, y,$ and z axes, respectively. They will be defined by $l, m,$ and n, where l is the cosine of the angle with the x-axis, etc. For a segment of length Δs with one end at the origin and the other at the point $(\Delta x, \Delta y, \Delta z)$, $l = \Delta x/\Delta s$, $m = \Delta y/\Delta s$ and $n = \Delta z/\Delta s$.

B. *Elemental growth-rate in volume per unit volume.* Consider an element of volume in the shape of a rectangular parallelopiped. For convenience choose the coordinate axes so that the origin is fixed at the vertex of the element and the three edges

meeting at that vertex initially lie along the positive halves of the axes respectively, as in figure 1B. Let the lengths of the sides be Δx, Δy and Δz. Then the volume is $\Delta x \cdot \Delta y \cdot \Delta z$.

As growth occurs the element will be deformed. The velocity of the vertex originally at $(\Delta x, 0, 0)$ may be obtained as follows. Due to the choice of the coordinate system, the velocity of the point at the origin is zero. Then the x-component of the velocity at $(\Delta x, 0, 0)$ will be given by $\Delta x \delta \dot{x}/\delta x$ + higher order terms in Δx; the y-component by $\Delta x \delta \dot{y}/\delta x$ + higher order terms in Δx; and the z-component by $\Delta x \delta \dot{z}/\delta x$ + higher order terms in Δx. Then in a short interval dt the coordinates of the point become

$$\left(\Delta x + \frac{\delta \dot{x}}{\delta x} \Delta x dt, \frac{\delta \dot{y}}{\delta x} \Delta x dt, \frac{\delta \dot{z}}{\delta x} \Delta x dt\right).$$

Similarly the point originally at $(0, \Delta y, 0)$ moves to

$$\left(\frac{\delta \dot{x}}{\delta y} \Delta y dt, \Delta y + \frac{\delta \dot{y}}{\delta y} \Delta y dt, \frac{\delta \dot{z}}{\delta y} \Delta y dt\right).$$

The point originally at $(0, 0, \Delta z)$ becomes

$$\left(\frac{\delta \dot{x}}{\delta z} \Delta z dt, \frac{\delta \dot{y}}{\delta z} \Delta z dt, \ \Delta z + \frac{\delta \dot{z}}{\delta z} \Delta z dt\right).$$

Thus the rectangular element is deformed into a non-rectangular parallelopiped. The volume of the new figure can be computed by standard methods of analytic geometry or vector analysis, and is found to be, to first order terms in dt, $\Delta x \Delta y \Delta z + \left(\frac{\delta \dot{x}}{\delta x}\right.$ $+ \frac{\delta \dot{y}}{\delta y} + \frac{\delta \dot{z}}{\delta z} \left.\right) \Delta x \Delta y \Delta z dt$. Thus the increment in volume is $\left(\frac{\delta \dot{x}}{\delta x} + \frac{\delta \dot{y}}{\delta y}\right.$ $+ \frac{\delta \dot{z}}{\delta z} \left.\right) \Delta x \Delta y \Delta z dt$, and the *increment in volume per unit volume per unit time* is $\frac{\delta \dot{x}}{\delta x} + \frac{\delta \dot{y}}{\delta y} + \frac{\delta \dot{z}}{\delta z}$. If Δx, Δy, Δz and dt be made vanishingly small, the neglected higher order terms also vanish, and the above expression thus is the exact expression for elemental increase in volume per unit volume per unit time. In vector analysis it is known as the *divergence of velocity function*, and is denoted by $\nabla \cdot \mathbf{v}$ where $\mathbf{v}$ is the velocity vector.

In the case of growth in a plane the third term is zero, and the expression becomes that for *increment in area per unit area per unit time* (area specific growth-rate of section III) : $\frac{\delta \dot{x}}{\delta x} + \frac{\delta \dot{y}}{\delta y}$.

C. *Elemental growth-rate in length per unit length.* Consider a segment of length Δs whose end points may be taken as

(0, 0, 0) and (Δx, Δy, Δz); in this case it is convenient to take Δs, Δx, Δy, and Δz as the changing values of the quantities rather than the fixed values of the particular instant under consideration. Then $(\Delta s)^2 = (\Delta x)^2 + (\Delta y)^2 + (\Delta z)^2$;

$$2\Delta s \frac{d\Delta s}{dt} = 2\Delta x \frac{d\Delta x}{dt} + 2\Delta y \frac{d\Delta y}{dt} + 2\Delta z \frac{d\Delta z}{dt};$$

$$\frac{1}{\Delta s}\frac{d\Delta s}{dt} = \frac{\Delta x}{(\Delta s)^2}\frac{d\Delta x}{dt} + \frac{\Delta y}{(\Delta s)^2}\frac{d\Delta y}{dt} + \frac{\Delta z}{(\Delta s)^2}\frac{d\Delta z}{dt}.$$

Since the origin is fixed at one end of the segment, $\dfrac{d\Delta x}{dt} = \dfrac{\delta \dot{x}}{\delta x}\Delta x$
$+ \dfrac{\delta \dot{x}}{\delta y}\Delta y + \dfrac{\delta \dot{x}}{\delta z}\Delta z$; $\dfrac{d\Delta y}{dt} = \dfrac{\delta \dot{y}}{\delta x}\Delta x + \dfrac{\delta \dot{y}}{\delta y}\Delta y + \dfrac{\delta \dot{y}}{\delta z}\Delta z$; $\dfrac{d\Delta z}{dt} = \dfrac{\delta \dot{z}}{\delta x}\Delta x + \dfrac{\delta \dot{z}}{\delta y}$
$\Delta y + \dfrac{\delta \dot{z}}{\delta z}\Delta z$. Substituting these equations in the expression $\dfrac{1}{\Delta s}\dfrac{d\Delta s}{dt}$, and using the expressions for the direction cosines of the segment, the result can be written:

$$\frac{1}{\Delta s}\frac{d\Delta s}{dt} = l^2 \frac{\delta \dot{x}}{\delta x} + lm \frac{\delta \dot{x}}{\delta y} + ln \frac{\delta \dot{x}}{\delta z}$$

$$+ ml \frac{\delta \dot{y}}{\delta x} + m^2 \frac{\delta \dot{y}}{\delta y} + mn \frac{\delta \dot{y}}{\delta z}$$

$$+ nl \frac{\delta \dot{z}}{\delta x} + nm \frac{\delta \dot{z}}{\delta y} + n^2 \frac{\delta \dot{z}}{\delta z}.$$

If now the chosen value of Δs be made vanishingly small, the initial direction of the segment being unaltered, the neglected higher order terms in Δx, Δy, Δz become vanishingly small and the expression on the right of the above equation is the exact expression for *elemental growth in length per unit length per unit time* (the specific growth rate in length). Denote this quality by L.

Since L involves the direction cosines l, m, and n, it follows that the specific growth rate in length may in general vary with the direction from the point which is being considered. Thus a small sphere centered at the point and growing for a short time would not in general remain a sphere, but would be somewhat deformed. To study the distribution of values of L, let $L = 1/r^2$, $rl = X$, $rm = Y$, $rn = Z$, and substitute in the equation for L. The resulting equation is that of a central quartic surface in X, Y, and Z, the radius of the surface being r. (In case L is negative, so that r is imaginary, take $L = -1/r^2$.) The properties of these surfaces are well known. When r has a maximum, L has a minimum and vice versa.

In the case of growth in a plane, the expression becomes

$$L = \cos^2 \theta \frac{\delta \dot{x}}{\delta x} + \cos \theta \sin \theta \frac{\delta \dot{x}}{\delta y} + \sin \theta \cos \theta \frac{\delta \dot{y}}{\delta x} + \sin^2 \theta \frac{\delta \dot{y}}{\delta y} ,$$

where $\cos \theta = l$ and $\sin \theta = m$. This equation was used in obtaining the specific growth rates in length in section III.

To determine the directions of maximum and minimum rates for plane growth, differentiate L with respect to θ, equate the result to zero and solve for θ. Calling the resulting values of the angle θ_m, it follows that $\tan 2\theta_m = \left[\dfrac{\delta \dot{x}}{\delta y} + \dfrac{\delta \dot{y}}{\delta x}\right] \Big/ \left[\dfrac{\delta \dot{x}}{\delta x} - \dfrac{\delta \dot{y}}{\delta y}\right]$. Since $\tan 2\theta_m$ has a period $\pi/2$ in θ_m and since values of θ_m corresponding to maximum L must alternate with those corresponding to minimum L, it follows that the directions of maximum and minimum L are at right angles to each other. Necessary conditions for L to be constant with respect to θ are those making $\tan 2\theta_m$ indeterminate; then $\dfrac{\delta \dot{x}}{\delta y} + \dfrac{\delta \dot{y}}{\delta x} = 0$; $\dfrac{\delta \dot{x}}{\delta x} - \dfrac{\delta \dot{y}}{\delta y} = 0$. Evidently they are sufficient. They are, of course, the Cauchy-Riemann conditions that the complex quantity $\dot{x} + i\dot{y}$ be an analytic function of $x + iy$.

If L is a constant at a point in three-dimensional space, it must be constant in each plane parallel to the coordinate planes; then the Cauchy-Riemann equations must hold in each such plane. Consequently, necessary conditions for constant L are $\dfrac{\delta \dot{x}}{\delta x} = \dfrac{\delta \dot{y}}{\delta y}$ $= \dfrac{\delta \dot{z}}{\delta z}$; $\dfrac{\delta \dot{x}}{\delta y} + \dfrac{\delta \dot{y}}{\delta x} = \dfrac{\delta \dot{y}}{\delta z} + \dfrac{\delta \dot{z}}{\delta y} = \dfrac{\delta \dot{z}}{\delta x} + \dfrac{\delta \dot{x}}{\delta z} = 0$. Evidently the conditions are also sufficient.

There is a simple relation between the growth rate in length per unit length and the growth rate in volume per unit volume at a point. Let L_x denote the value of L in the direction parallel to the x-axis, L_y the value in the direction parallel to the y-axis and L_z the value in the direction parallel to the z-axis. Then $L_x = \delta \dot{x}/\delta x$, $L_y = \delta \dot{y}/\delta y$, $L_z = \delta \dot{z}/\delta z$, and the volume rate $\nabla \cdot \mathbf{v} = L_x + L_y + L_z$. Since $\nabla \cdot \mathbf{v}$ is invariant with respect to a rotation of the axes (as is known from vector analysis) it follows that the volume rate equals the sum of the linear rates in any three mutually perpendicular directions.

D. *Technic of analysis of the tobacco leaf.* The methods used in applying the general formulae to Avery's drawings of the tobacco leaf are not sufficiently general to warrant detailed description. They will be sketched briefly, as suggestive of procedure for similar cases.

In place of calendar time (which was not available to us) the length of the central axis included between the extreme cross markings was chosen as the time variable. This procedure invalidated direct comparisons between rates in different stages, but did not affect ratios between rates in the same stage, or the comparison of such ratios for different stages. It was found that the coordinates of the moving points (intersections of the lines of the network) were very nearly linear functions of this time variable. Slight variations from linearity were observable, but the limitations of the data, mentioned above, made unjustified the extra labor of taking the variations into account.

Each coordinate of each point in turn was graphed against the time variable, and the slopes of the resulting lines were taken as the quantities $\dot{x}$ and $\dot{y}$. These quantities were next represented as functions of the coordinates (x_o, y_o) of the first stage. Then the values $\dot{y}$ for points along the central axis were plotted against the initial distance of the points from the origin, which was taken at the intersection point on the axis nearest to the base of the leaf. The slope of the resulting curve at each point was the value of $\delta\dot{y}/\delta y_o$ at the point. In a similar manner the quantities $\delta\dot{x}/\delta x_o$, $\delta\dot{x}/\delta y_o$, $\delta\dot{y}/\delta x_o$ and $\delta\dot{y}/\delta y_o$ were determined. The slopes were measured with a Tangentmeter.

In the initial stage the quantities $\delta\dot{x}/\delta x_o$ etc. were identical with $\delta\dot{x}/\delta x$, etc. required in the growth-rate formulae. Direct substitution in the formulae was therefore made to determine at each point the growth-rate in area per unit area, and the directions and values of maximum and minimum growth-rate in length per unit length.

For determination of $\delta\dot{x}/\delta x$ etc. for later stages it was necessary to solve linear equations giving the quantities in terms of $\delta\dot{x}/\delta x_o$, etc. The equations were fairly simple, due to the linear relations between the coordinates and the time variable. The resulting $\delta\dot{x}/\delta x$, etc. for each stage were then substituted in the general growth-rate formulae.

The contour lines for growth-rate in area per unit area were located by determining the intersections of each contour with each line of the network, by interpolation between adjacent points of intersection of the network; the points thus determined were joined by smooth curves.

GEOGRAPHICAL VARIATION AND RACIAL
STRUCTURE OF ARGYNNIS CALLIPPE
IN CALIFORNIA

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INTRODUCTION

SEVERAL species of butterflies in the western part of
North America present excellent material for the study
of the origin and composition of geographical races as
well as for an interpretation of the genetic and ecologic
significance of racial or subspecific variations. *Argynnis
callippe* (Lepidoptera: Nymphalidae) is a butterfly of
wide distribution. It is also very abundant from the
point of view of number of individuals per population.
These populations are partially (or completely) isolated
one from the other by areas more or less large in which
breeding conditions are unfavorable. The species is also
relatively non-migratory, individuals tending to stay in
the region of their early growth. These factors, as well
as a great climatic variability in the region, seem to have
been responsible for an immense variety of geographical
differences between the populations. No attempt will be
made to give a complete systematic account of this group
as the time and funds have not been available for tracing
the complete history of names and types. A systematic
account is worthless without this information. Instead,
the emphasis will be placed upon the description of varia-
tion and distribution which really is greatly needed in
this common and highly variable group, since this group
has not yet received organization into a logical biological
arrangement. In a special section suggestions as to use
of names is given. Difficulties for the taxonomist in
determination of specimens are to be expected in a group
such as the Argynnis, where all species appear so much
alike and without good characters for designation and

comparative description. Parallel variation between species is an abundant feature of the Argynnis as in the closely related Melitaea and other butterflies. This feature makes, for the systematist, great difficulties. Different species will appear more like one another in the same region in color and size characters than the members of the same species in different localities (Hovanitz, 1941). Only by means of a dynamic evolutionary concept, a concept of varying gene frequencies in populations and of ecologic population mechanics does it seem possible that the variation of this species can be understood in its fullest. These populations are not uniform; they are not to be expected to be so. It is deplorable that some systematists shun the variant specimen, and eliminate it from their series because it does not follow exactly the description of their type individual.

The variation of most native species of butterflies in California is best studied by relating the variability to the topographic and ecologic features of the country. California may be described roughly as a large central valley about 450 miles long and about 60 miles wide, bordered on the west by a coastal series of ranges split into small valleys and hills, bordered on the east by the very high Sierra Nevada range, closed on the north by a connecting range and closed on the south by a curvature of the lengthwise ranges. This, of course, is only a rough description but will serve to illustrate the main features of butterfly distribution, the means of origination and the maintenance of the differentiation into local and extensive geographical races.

For the type of description which is to follow it would be foolhardy to attempt to adhere to a commonly accepted view that the type locality of a species or race or subspecies is the center of its origin or the center of its distribution. As is now becoming increasingly evident, the type locality is nothing more than the place where the first collector happened to find the different looking individual to which was given a new Latin name. Such a
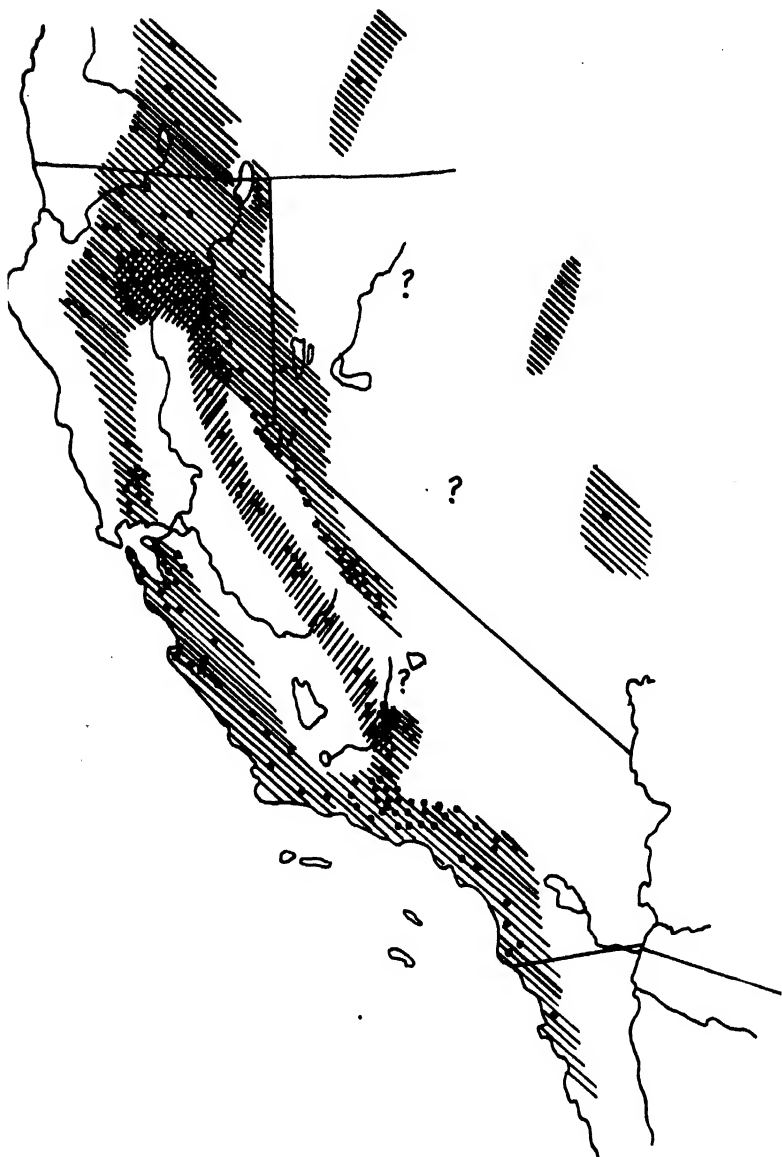
FIG. 1. Map showing the distribution of the characters silvered spots (shading slanting up to the left), and cream-colored spots (shading slanting up to the right). Areas unshaded represent regions from which the butterfly is unreported, and for the most part this means it is absent. Question marks mean that no search has been made to determine the presence of the butterfly in this area. This map also shows the distributions of buff

locality may have been and often was on the zone of inter-
gradation between two variation gradients where hybridi-
zation and the intermixing of different genes had pro-
duced a very heterogeneous population. Many old names
are, therefore, to-day difficult of application, especially
where the type series contained individuals now known
to belong to two or more geographic races. Such zones
of intergradation are very common and are of immense
importance for use in evaluating the interrelationships
of geographical races. They are, however, difficult to
correlate with names.

South Coast Range Populations[1]

Fortunately, the type locality of the individuals upon
which the name *callippe* was based forms a very con-
venient starting point from which to describe the distribu-
tion; it is at the end of a geographical gradient where a
topographical barrier appears to prevent intergradation
with the next closely related gradient. San Francisco,
at the end of the arm of land separating the San Fran-
cisco Bay from the Pacific Ocean, is one of the most north-
ern localities known for the variation gradient which
extends from this place, Berkeley and Mt. Diablo through
the south coast range, coastal southern California and
into Lower California. This gradient is characterized
primarily by a light-colored band (without distinct bound-
aries) running down the central portion of all wings.

coloring on the under side of the wings, for cream-colored spots and buff-
coloring seem to be related, as also, silvered spotting and yellow coloration.
The dots represent known localities of the butterfly. These have not been
reproduced on the successive maps. To synthesize character-combinations of
a population in any given place it is only necessary to mentally superim-
pose each of these first five maps over one another. Then for general
intensity of coloration, map six can be consulted. Local variation of various
types may also serve to affect the results obtained from the general maps, but
this should not be excessive. An unknown population can thus, within rea-
sonable limits, be hypothetically determined.

[1] The description of the variation should be followed by reference to the
maps which are arranged according to character. For specific localities, more
detailed maps not shown here must be consulted.

(See Hovanitz, 1941, Fig. 4.) An occasional individual does not show this. The gradient is not truly a straight line of variation because it has width as well as length; variation occurs from east to west across the coastal ranges as well as north and south along them. At San Francisco (formerly) and San Mateo County this gradient reaches its maximum of darkness. This darkness is produced by a widening of the pattern elements of melanin, by a heavier scaling of melanin-colored basal scales on all wings and by a darkening of the yellow-brown pigment (Group Two-a, Hovanitz, 1941). At Berkeley, 15 to 20 miles inland, under a lesser influence of the coast, the darkening is not as extreme, though occasional individuals are quite dark. South from North Berkeley Hills (Contra Costa Hills), in the San Leandro Hills, the populations become increasingly lighter in color. One particular population east of Hayward has surprisingly light-colored individuals. In between, in East Oakland (Sequoia Park) there are intermediates. Farther south toward Sunol and Calaveras Valley the butterflies are likewise very light in color. It is possible to correlate this color change with the cooling produced by the ocean breezes and fog at Berkeley opposite the Golden Gate, together with a decrease of this climatic effect south through the hills where the San Mateo Ranges and the Santa Cruz Mountains serve to block off the ocean cooling from the interior. At Mt. Diablo, the butterflies are also very pale in color and in size are quite small. This locality is 40 miles inland and far from much fog-cooling. At Carmel Valley, the Santa Lucia Ranges and down to the Santa Inez Ranges the populations are quite variable, depending upon the local climatic conditions. In the interior of the Santa Lucia Ranges occurs one of the lightest colored populations—almost white, but this is continuous with others which are quite dark in color. In the region, Santa Inez Mountains to the San Gabriel Mountains, the populations tend to have a higher percentage of individuals partially or totally lacking the

central light band.   This increases east through the
Tehachapi Range, where connection is made with the
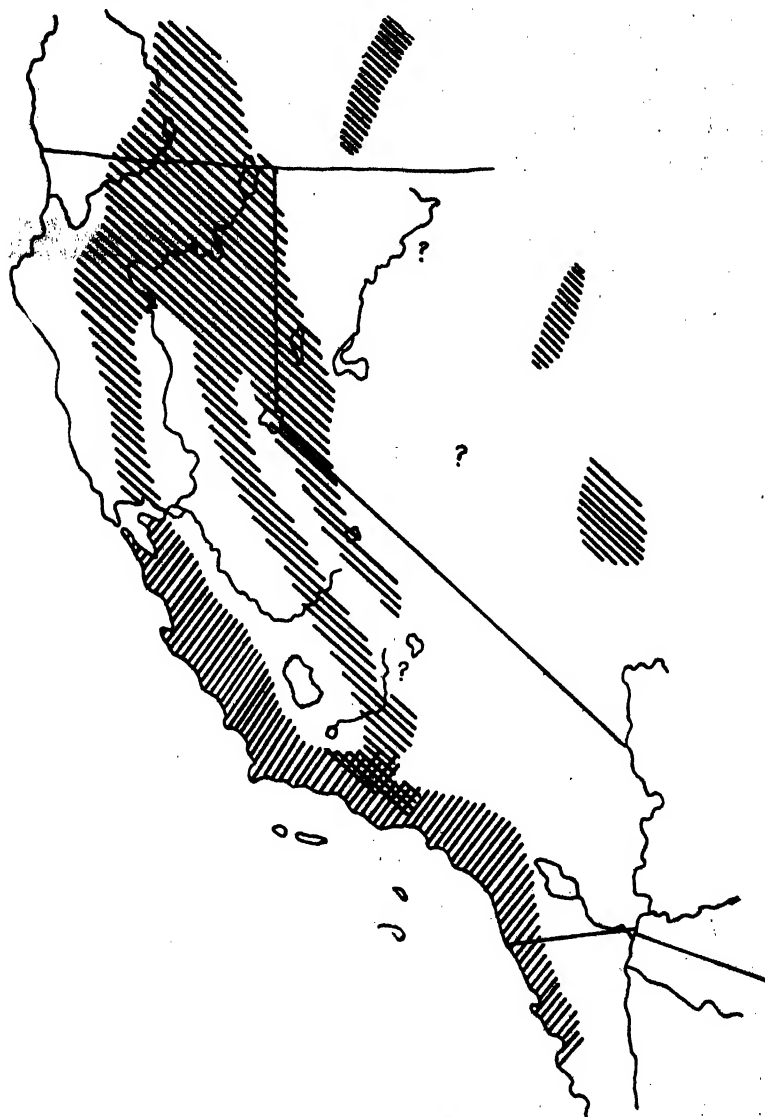
Fig. 2. Map showing the distribution of the light-colored band on the
upper surface of the wings (shading slanting up to the right) as compared
with a uniformly colored red-brown upper surface.

Sierra Nevada races, which are to be considered later.
South in the coastal hills of Southern California and

Lower California there is as much local variability as is present in the San Francisco Bay area, but here the blacker individuals appear to be found in the foothills of the San Gabriel Range. This is likewise true with respect to some Melitaea (*M. chalcedona* especially) and may be correlated with the colder winters and higher rainfall along the western face of this range. No *Argynnis callippe* has ever yet been taken east of the Southern California mountains in the Mohave and Colorado Deserts proper or high in these mountains. It is likewise completely absent from the flat valley areas of the Great Valley of California, from the San Francisco Bay-Santa Clara Valley flat areas, the Livermore Valley, the Salinas Valley, the Carrizo plains and adjoining arid hills, the Santa Maria and Cuyamaca valleys, the valleys and plains of Southern California (Los Angeles Plain, San Gabriel Valley, Santa Clara River Valley, San Fernando Valley, San Bernardino Valley, etc.), being found only in the hilly country—and only locally there. It was perhaps absent from these places even before the cultivation induced by present civilization destroyed the native vegetation. In many places, it is rapidly becoming exterminated by destruction of Viola by the turning over of the soil and now is found only in rocky places for the most part.

Characteristic points concerning the pattern and color of the *callippe* are listed as follows: (1) Silver spots always present on underside hind wings and apex of fore wings; (2) brown scaling between median row of spots on hind wing and base of the wing, no green scaling present; (3) yellow band between marginal spots and median row of spots wide and clearly distinguishable; (4) light-colored band across all wings on upper side; (5) areas on the upper side of the wings, immediately above the silver spots, tend to appear light in color, though this is true more of some populations than of others.

It should be mentioned that dark- or light-colored populations are found along this distributional area anywhere

at (it seems) climatically suitable areas. There appears in this way similar-appearing, completely unconnected local populations separated from one another by other different populations. Climatic selection would appear to be the active agent in the origination or evolution of the local differences and possibly also of the larger series of variations.

WESTERN SIERRA NEVADA POPULATIONS

This coastal gradient connects with the western Sierra Nevada variation gradient through the Tehachapi Mountains. It will be best to describe the Sierra Nevada gradient in general first, before describing the specific state of the zone of intergradation. In the area from the northern Sierra Nevada and southern Cascade Ranges (region west of Lassen Peak and the "Juba" Mountains) to the southern Sierra Nevada the butterflies are characterized by a fairly uniform upper-surface yellow-brown color with a lack of the light band present in the coastal gradient. Also, the spots on the under surface of the wings are not silvered but rather are cream or buff-colored. The band between the marginal spots and the median row of spots is also buff-yellow or just buff instead of yellow as in the coastal gradient. The areas on the upper surface of the wings above the silvered spots are not light colored. The areas between the spots other than the above band on the under side of the hind wing are brown scaled but apparently more uniform than in the coastal gradient. That is, the coastal material has a tendency to have the area interspersed with yellow scaling. Superimposed upon this general description is a variation in darkness and lightness of all the pigmentation from north to south respectively. Melanin pigmentation is increased wherever present, in the width of the pattern elements and in the scaling of the wings at the bases. The brown pigment of the underside, the yellow-brown pigment of the upper side as well as the intensity of the yellow-buff is increased or darkened in the north as compared with the south. The gen-

eral elements remain the same, however. The life-zone preference of the insect in the western Sierra Nevada is the area between the Upper Sonoran and the Transition, ranging from elevations in the north (Shasta, "Juba" Mountains) from 2,000–3,500 feet to 4,000–5,500 feet in the south (Greenhorn Mountains). Through the length of the territory from the Greenhorn Mountains (one of the southern ends of the Sierra Nevada) to the "Juba" Mountains area, the gradient is almost a perfect line with little width. The Central Valley of California is a barrier to intergradation to the west and the boreal regions of the Sierra Nevada are a barrier on the east. Only to the north, in the vicinity of the Yuba River and thence to the Shasta country is there possibility of much movement eastward; here the mountains are relatively low. Passes are not far above the limit reached by the butterflies' preferences as to climate, and, in part, the semi-desert area of the Great Basin invades the volcanic lands of Lassen and Modoc Counties. Here the species is found continuously from the Central Valley foothills of California through to the Great Basin. This is the country where the Basin territory is partly drained by the Pit River, one of the few outlets through the mountain barrier. Phylogenetically, this low opening permits of the exchange of genes from the Basin populations with the coastal or western ones. The effect of this on the variation in the region is great and will be described a little later.

Southern Zone of Intergradation

It remains to describe the connection of the southern end of the range of the western Sierra Nevada gradient given as the Greenhorn Mountains with the coastal gradient in the region where the Sierra Nevada meets the coastal ranges. This is done in a series of steps across the Piute Mountains, the Tehachapi Mountains and the Sierra Madre Range. From the Santa Monica Mountains on the coast, where the typical *callippe* of this region

lives, it is found that in going inland (Charlton Flat, Mint Canyon, Bouquet Canyon and "Ridge Route") the lightness of all colors increases. The butterfly becomes
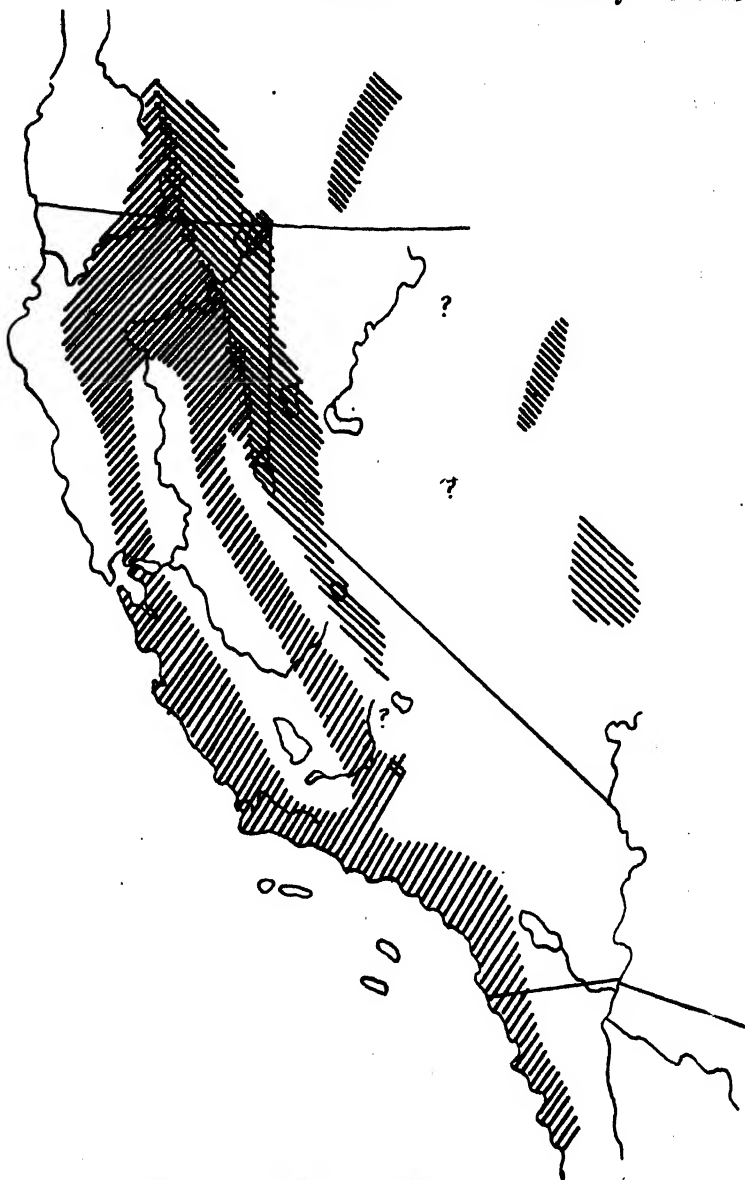
FIG. 3. Map showing the distribution of green pigment on the under surface of the wings exclusive of the marginal band (shading slanting up to the left), as compared with the lack of the pigment.

smaller and the light-colored band and spots on the upper surface of the wings tend to become obliterated, leaving a more uniformly colored wing surface such as is present in the western Sierra Nevada gradient. However, the yellow-brown color is very much lighter than in the latter and the band on the under side of the hind wings is still yellow; the spots are still always fully silvered. The tendency toward these conditions is the more marked the farther from the coast and the farther into the Tehachapi Range the populations exist. In the Tehachapi Range, the butterflies are very lightly colored and the band on the upper surface of the wings is rare; the spots are still silvered. At Havilah, Piute Mountains, the population consists of some silvered, some unsilvered and some intermediate spotted individuals (this is the type locality of *macaria* Edws.); the exact frequency of these types is not known, but there is a high percentage of silvered and unsilvered present. In the Piute Mountains, the first sign of a segregation into an eastern type and a western type of variation is observed. At Kelso Valley, on the eastern side, the tendency is toward silvered spots (23 silvered and two intermediates obtained); whereas on the western side at Havilah, unsilvered are very common, though comparative figures are not available. At Kelso Valley, a tendency toward the appearance of green coloring on the under side of the hind wings is apparent in a few individuals. The relation of this to the distribution of the Great Basin form *nevadensis* will be discussed later. In the Greenhorn Mountains, the segregation into a silvered population on the eastern side of the summit and an unsilvered one on the western side is decidedly apparent, though mixing occurs toward the south, where the populations unite. At Cedar Creek (5,000 feet) on the western side of the summit (7,000 feet) the distribution of variation in a small series was: 10 not silvered or very slightly so, 8 intermediate and 4 well- or fairly well-silvered. On the opposite side of the range at an elevation of 5,500 feet, 14 silvered, two intermediates and no

unsilvered were obtained.[2]   These numbers are not large, but they are suggestive of a segregation into distinct populations here, even though the distance between the localities was not more than ten miles airline, and they are separated by a *callippe*-uninhabited area very small in extent. Of course, it can not be assumed that such difference could long exist without a powerful selection for the types or, more certainly the case in this instance, a flow of genes from the centers of populations of each of the silvered and unsilvered forms into this zone of intergradation. Thus the gap is bridged between the western Sierra Nevada gradient and the coastal populations in the south. It should be noted that the gene frequencies have not changed at the same place. Silvering changed at the Greenhorn Mountains; north were unsilvered-gene bearing populations, south the silvered-gene bearing populations. The upper surface pattern made its change in the area from the Tehachapi Mountains to the Bouquet Canyon or the Santa Susana Hills area. In addition, it is observed that the general coloring from dark to light made its changes from north (Shasta and Lassen) to south (Tehachapi) gradually and with no apparent relation to the other changes. It would appear that the genes affecting these characters segregate independently of one another and do so in different geographical regions. However, later we shall see that there is a possibility that silvering and buff coloration are in some way directly related. These both change in the Greenhorn Mountains.

The Northern Populations

As has been mentioned, the northern Sierra region is low, allowing genes to be transmitted between the Basin populations and the western Sierra Nevada populations. The former populations have silvered spots, as do populations farther north in Oregon. Consequently, the re-

[2] The collections at Cedar Creek and eastern side Greenhorn Mountains as well as those at Kelso Valley, Piute Mountains were made on the same day. Much more material from other years is available from the Greenhorn Mountains but is not segregated into specific localities.

gion is mixed, with populations tending to have unsilvered spots on the areas bounding the valley and increasing silvering north and east. This mixture has led to great confusion in the names given to forms from this region. As the Basin populations have a lighter general coloring than the western populations, there is also a transition of this variability through here. The northern limit of the unsilvered populations appears to be the Shasta, Trinity and Siskiyou region. South in the North Coast Range, north into Oregon, east into the Great Basin, silvering is the rule. Only south into the western belt of the Sierra Nevada does unsilvering extend. South through the North Coast Range as far south as Sonoma, Napa, etc., the distribution of this form occurs. The general ground color here is reddish-brown, quite dark in the north (*rupestris*) but becoming lighter to the south (*liliana*). The arms of the San Francisco Bay, the San Pablo Bay and the Carquinez Straits seem to isolate this gradient from the northern end of the south coast gradient, which reaches its northernmost locality in the Berkeley Hills. Some populations in Napa County appear to have individuals seeming to be intermediates (Huichica Creek); the relative abundance of these is very low. This gradient from the Siskiyou, Humboldt and Trinity Mountains to the San Francisco Bay may be characterized then by: (1) a quite uniform red-brown ground color without the light band and spots typical of the south coast form; (2) all heavily silvered spots; (3) a relatively dark color of melanin, red-brown and yellow pigment. There is slight north-south variation in darkness. This gradient has in common with the south-coast gradient the silvered spots, and the yellow colored band on the under side hind wings. It has in common with the western Sierra gradient, the uniform upper surface ground color and the reddish-brown tint to this color rather than the yellowish-brown tint as with the south coast gradient. It should be noted that the only characters changing in the region of the northern end of the valley between the Sierra Nevada
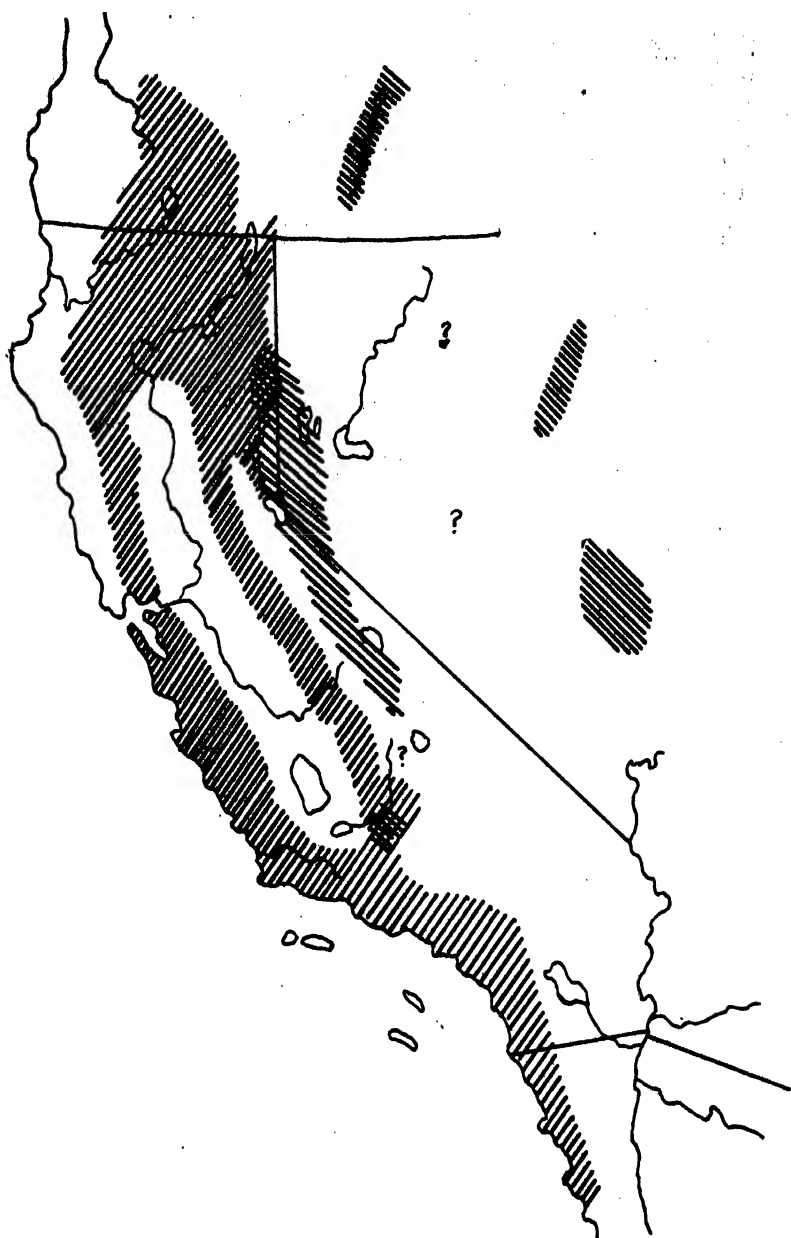
Fig. 4.   Map showing the distribution of brown pigment between the spots on the under side of the hind wings (shading slanting up to right) as compared with the lack of this pigment.
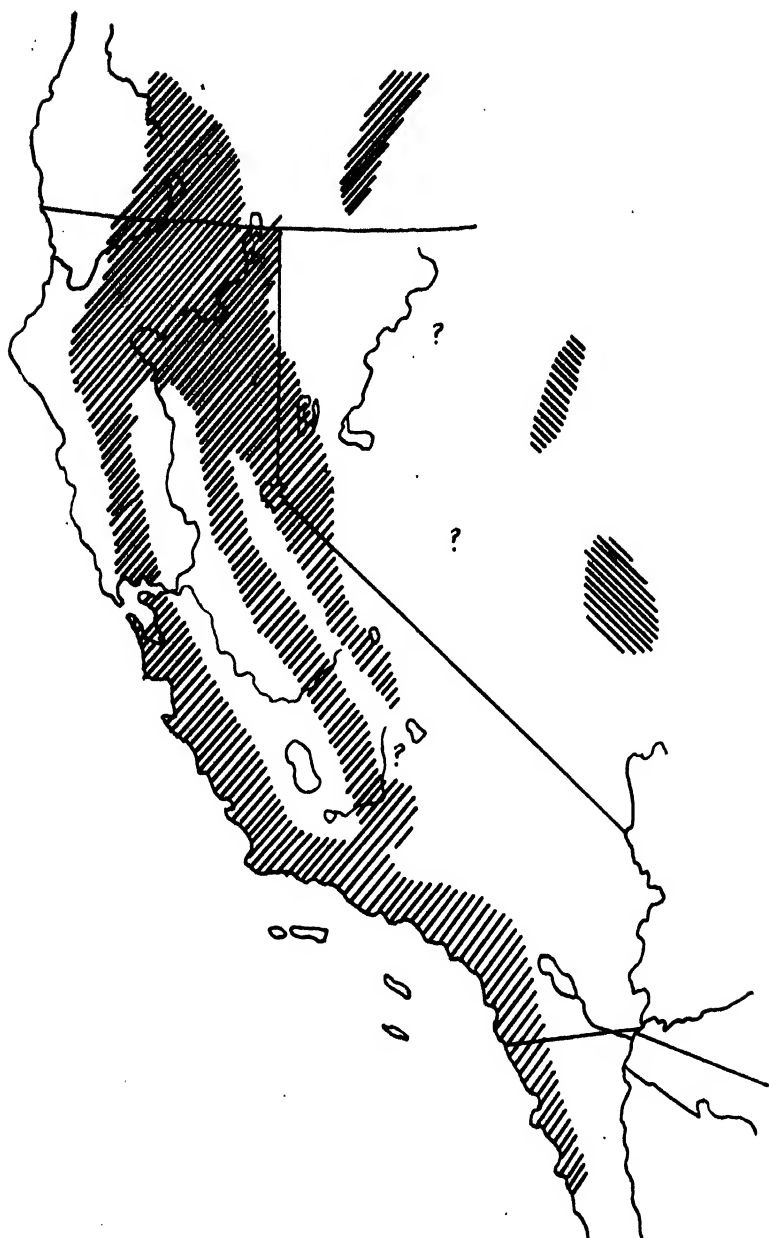
Fig. 5. Map showing the distribution of green pigment scales which occur in the yellow bands on the under side of the hind wings, (shading slanting up to the left) as compared with the lack of this pigment.

and the north coast gradients is the change from unsilvered in the former to silvered spots in the latter, and the change from buff pigment on the underside to yellow, respectively. Information as to what happens from here north into Oregon is scanty.

Going eastward through the low mountains in the northern Sierra Nevada, the populations become increasingly silvered (Downieville, etc.). The Great Basin populations (*nevadensis*) are entirely silvered. These populations in California are characterized usually by an upper surface coloration much like western Sierra Nevada populations, that is, with an absence of the central light band and having a uniform ground color; this coloration is fairly light and the melanin is not very extensive. The band on the under side of the hind wings is yellow, not buff, as is the rest of the light pigment on the under side of the wings. In the northern populations, desert Oregon, Modoc and Lassen Counties, there is present brown pigment between the spots on the under side of the hind wings. This decreases southward and appears to be absent from at least Virginia City, Nevada, south through more than ten known localities to Bishop, Inyo County, California. In all populations of this area, the wing is also suffused with green scaling on the same part of the wings. This green is present likewise around the basal side of the border silver spots and on the apex. In the north both the brown and green is present; in the south the green only, on a yellow ground. The yellow band on the hind wing appears to be constant only along the western-most of the Great Basin populations. Eastward, it is suffused with green in most cases (*meadii*). As has been previously mentioned, between the populations of the Basin form and the western Sierra Nevada form, a zone of intergradation occurs. Here, the populations become silvered eastward and do so before they obtain the green on the underside. Thus, areas are formed in which the butterflies are identical with those from a totally unrelated region, namely, the north Coast Range.

It would not appear likely that the populations at these two places, isolated from each other in this way, could have had a common origin. Instead, it seems more plausible to assume that by chance a similar combination of these color genes has taken place. If so, there is parallel evolution in two different places. The circumstance noted, that the point of change-over from silvering to non-silvering seems to bear no relation to the place of change-over from green scaling to non-green scaling, is additional information to show that the genes involved in the production of these geographical races are for the most part free to be synthesized into any combination whatever. Although localities for the green-under-side form are at present unknown south of Bishop on the eastern escarpment of the Sierra Nevada, the appearance of slight green pigment on a few individuals from the Piute Mountains, Kern County, leads one to think that there may be a connection. However, since the locality of the latter is on the edge of the desert (Kelso Valley) it may be a new development of the genes in the population, with no relation to the northern, probably unconnected, form.

SUMMARY OF THE VARIATION AND DISTRIBUTION

Table 1 shows the distribution of these characters in given geographical areas.

It may be observed that the segregation of characters between these different regions is, except for one case, completely independent. This one case is that previously mentioned, non-silvered spots and buff coloration which is present (shown by minus sign) in the western Sierra Nevada gradient. This correlation may be due to linked genes, to a common physiological-developmental basis controlled by a single gene, or possibly to preference selection by a similar type of environmental agency; the first possibility seems the most remote, because a linkage of this sort should break down often enough to give many exceptions. The second seems most probable to the author. Whatever the developmental reason, the two

TABLE 1

	silvered spots present	light-colored band on upper side; also, often light above spots	yellow band on under side as contrasted to buff	brown pigment between spots	green pigment present	green pigment present in yellow band on under side	names applicable to the type of variation or region
South Coast Range gradient	+	+	+	+	−	−	*callippe, comstocki*
North Coast Range gradient	+	−	+	+	−	−	*liliana, rupestris*
Western Sierra Nevada gradient	−	−	−	+	−	−	*juba, inornata, laurina, macaria*
Sierra Madre-Greenhorn Mountains zone	+	−	+	±	−	−	*macaria, comstocki*
North Sierra Nevada intermediate zone	+	−	+	+	−	−	*juba, rupestris, inornata, liliana*
Modoc-Lassen and north ...	+	−	+	+	+	−	*semivirida, laura*
Eastern Sierra Nevada	+	−	+	−	+	−	*nevadensis*
Eastern Great Basin	+	−	+	−	+	+	*meadii*

characters seem to be present together in the same individuals as well as being present in the same general geographic region.

As noted before, two regions have a similar distribution of characters, namely, the north-coast range gradient and the intermediate north Sierra Nevada zone. Since the ground color of the latter is relatively light or intermediate brown in color, most butterflies from this region can not be distinguished from butterflies from the southern part of the north-coast range gradient (Napa and Sonoma Counties) without a long series. In the latter case, the aberrant individuals serve to differentiate the population; the aberrant individuals are more like their nearest relatives (in the intermediate north Sierra Nevada zone aberrancies are taken with a little green coloration and with silvering tending to disappear). In the southern part of the North Coast Range gradient, aberrancies tend to get a light band across the upper surface of the wings—like typical *callippe*.

"DARKNESS AND LIGHTNESS"

Besides the six apparently qualitative color variation types described above, which might be related to six (or five) specific genes, there is the type of variation which controls the darkness and lightness of all pigments on the insect, that is, the quantity of it. Genes controlling this type must act in development at such a time as to affect all pigments, that is, must be general influencers of pigment metabolism or deposition. The characteristics of the variation in this butterfly are an increase in intensity and area of the melanin pigment (Group 1), an increase in intensity of the red-brown or yellow-brown pigment (Group 2a) and likewise of the yellow pigment where present (Group 2b). The melanin increase in area comes from a widening of the pattern elements and a heavier scaling extending out from the base of the wings. Because of the light-colored band which is present in the South Coast Range gradient the specimens from this area are difficult to correlate with material from elsewhere which lack it. The map (Fig. 6) shows the distribution separately. It should be noted that this variation for the most part is independent of the other variation. It also seems to show a more direct correlation to specific environmental influences (see Hovanitz, 1941, for full data). The darker individuals and populations are present in regions of least light intensity, greatest moisture or humidity, lowest temperature and longest available growth period. The lighter occur under the opposite conditions. It is interesting that the populations at the north end of the South Coast Range gradient possess a much greater amount of melanin pigment than the populations at the southern end of the North Coast Range gradient. Since the South Coast Range gradient has a light-colored band (of Group 2a pigment) on the wings, it may be that the darkening of the wings can not take place with that pigment so long as the given gene is present and the color change then takes place in the melanin pigment (Group 1 pigment). North of the San Francisco Bay, the inhibitor
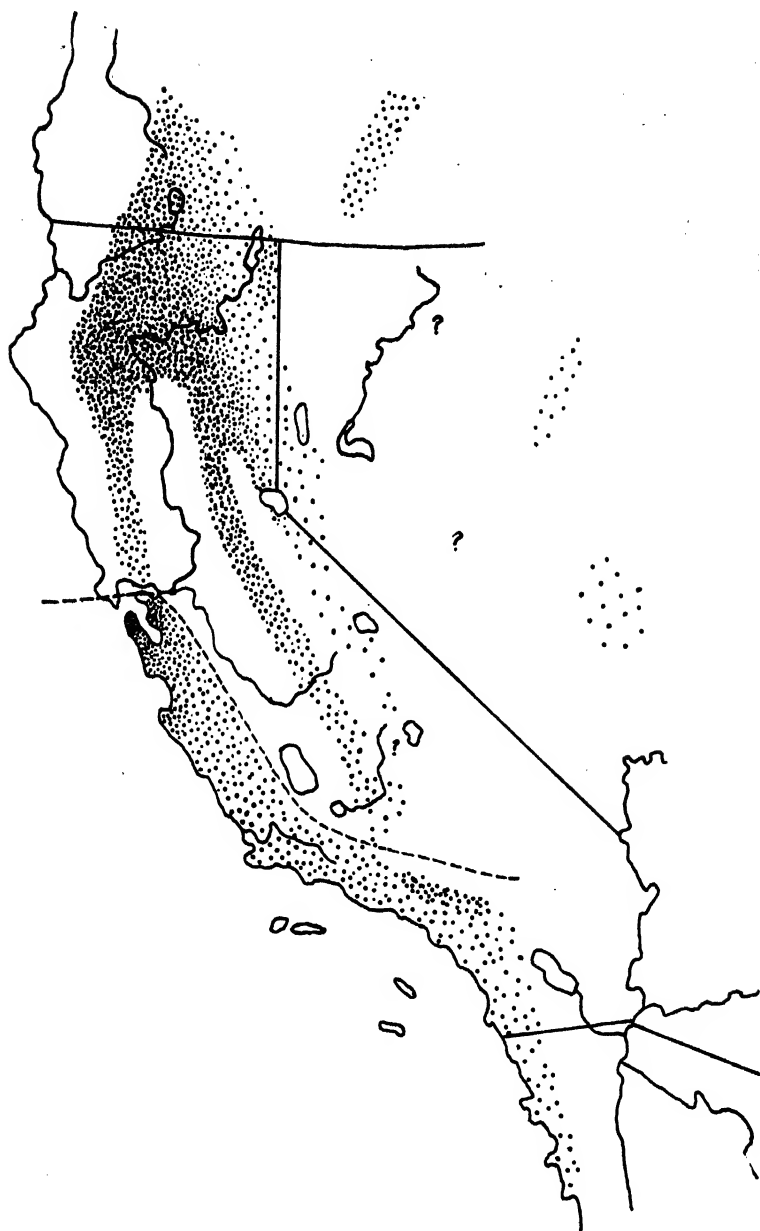
FIG. 6. Map showing by intensity of shading the darkness or lightness of the upper surface of the wings. The area above and to the right of the dashed line should be considered separately from that below and to the left because the light-colored band existent in specimens from the latter region interferes with the comparison between these regions.

gene (which controls the light-colored band) is not present; the Group 2a pigment is darker and the Group 1 pigment is lighter, it appears, to make up for this deficiency.

Other Ecological Correlations

The significance, if any, of silvered and unsilvered spots on the under surface of the wings is quite incomprehensible. In this species, as well as others in the genus, there seems to be no significant correlation between silvered spots or unsilvered spots and environmental factors. In general, but rather weakly so, silvering seems to be commoner in more southern regions than northern ones in the subfamily to which the Argynnis belong. Perhaps the coloration is correlated with the buff pigmentation above mentioned and carried along with it. But in this case, the buff coloration might have some significance.

The significance of the light band across the wings of the South Coast Range gradient possibly may be correlated with the southern location of the region. It has the most southern distribution in the species and would be expected to be light in color. Whatever the physiological developmental basis and foundations of the color differences, it might appear that any gene that controls a process in ontogeny that allows the animal to be better adapted to live in the environment of the south, will be selected for. Apparently such genes are correlated with a lighter color (Hovanitz, 1941). By means of several different methods, as earlier mentioned, a lighter color could have been obtained in this region, but apparently sufficient change was not possible of accomplishment by a continuation of the North Coast Range gradient. Such a relationship is not unknown in other species for example, *Coenonympha typhon california* is very light in color in this same approximate region and dark elsewhere.

The significance of the green pigment on the under surface of the wings is not too easy to correlate with any

specific environmental conditions. Other species in the same general region (Great Basin-Rocky Mountains), have such a coloration in this region which is found nowhere else in North America, namely, *Argynnis bischoffi-eurynome*,[3] *Argynnis edwardsi,* and possibly one other whose name is doubtful. Whether this coloration is of any physiological significance, directly or indirectly, with any ecologic factor is hard to imagine. The country is characterized especially by cold winters, hot and dry summers, and light-colored terrain.

The significance of the brown pigment between the spots on the under side of the hind wing is correlated with climatic conditions, as was discussed with the general pigment changes (Group 2a, Hovanitz, 1941). The absence of the pigment is found in arid, hot and southern regions, etc.

The names applied to this variation of *Argynnis callippe* in California are:

callippe Boisduval 1852. *Ann. Soc. Ent. Fr.,* 21: 302 (San Francisco, California).

comstocki Gunder 1925. *Entom. News,* 36: 8 (Los Angeles).

macaria Edws. 1877. *Field and Forest,* 3: 86 (Havilah, Kern Co., Calif.).

laurina Wright 1905. Butterflies West Coast, 138 (''Southern California Mountains'').

inornata Edws. 1872. *Trans. Am. Ent. Soc.,* 4: 64 (Downieville, Calif.).

juba Bdv. 1869. *Ann. Soc. Ent. Belge,* 12: 60 (''Juba mountains'').

laura Edws. 1879. *Can. Ent.,* 11: 49 (Nevada).

rupestris Behr 1863. *Proc. Calif. Acad. Nat. Sci.,* 3: 84 (Sierra Nevada).

liliana Hy. Edws. 1876. *Proc. Calif. Acad. Sci.,* 6: 170 (St. Helena, Napa Co.).

nevadensis Edws. 1870. *Trans. Am. Ent. Soc.,* 3: 14 (Virginia City, Nev.).

semivirida McD. 1924. *Can. Entom.,* 56: 42 (Aspen Grove, B. C., Canada).

meadii Edws. 1872. *Trans. Am. Ent. Soc.,* 4: 67 (Turkey Creek Junction, Colorado).

It is the author's suggestion that these names might be used in the following manner, it being understood that the types have not been examined:

[3] For some unknown reason, *bischoffi* is considered a race of *eurynome* in the latest check list. *Bischoffi* appears to be the oldest name available here: *Argynnis bischoffi* Edws. 1871. (1870), *Trans. Am. Entom. Soc.,* 3: 189, *Argynnis eurynome* Edws. 1872, *Trans. Am. Entom. Soc.,* 4: 66. The hyphened name is used here to designate the general species, since *bischoffi* is not well known.

Argynnis callippe callippe for the gradient extending from Berkeley and San Francisco on the north to Lower California on the south along the coast ranges. (*comstocki* as synonym).

Argynnis callippe laurina or *rupestris*, for the gradient extending from the mountains at the head of the Great Valley to the Greenhorn Mountains, *i.e.*, the unsilvered race. (*laura, laurina* or *rupestris, juba, macaria, inornata* being smaller entities if used at all).

Argynnis callippe liliana Hy. Edws., for the gradient extending from Northern California to San Francisco Bay in the North Coast Ranges.

Argynnis callippe nevadensis for the triangular gradient extending from British Columbia on the north to Bishop on the south, and eastward to the Front Range of Colorado.

These are major divisions, but if it is desired that smaller and easier-to-use units be organized the following is suggested:

Argynnis callippe callippe as above, bearing in mind the tremendous local variation which also might be further subdivided. In the latter case *comstocki* could be used for the south or many other names might be applied for local populations.

Argynnis callippe macaria for the heterogeneous mixture extending from the Sierra Madre to the Greenhorn Mountains. This is in some places quite constant and is intermediate between the western Sierra Nevada gradient and the coastal gradient.

Argynnis callippe laurina for the light-colored, southern individuals of the western Sierra Nevada unsilvered gradient.

Argynnis callippe inornata for the intermediate-colored silvered part of the western and northern Sierra Nevada zone.

Argynnis callippe rupestris for the area around the northern end of the Great Valley where the coloration is fairly uniform except for the mixing of the unsilvered and silvered.

Argynnis callippe liliana for the gradient extending from the zone designated *rupestris* to the San Francisco Bay. There is a change in darkness and lightness of ground color here.

Argynnis callippe nevadensis for the zone extending from Virginia City, Nevada, and Truckee, south to Bishop in which green is present and brown absent between the spots on the under side hind wings, and a yellow band is present.

Argynnis callippe semivirida for the zone extending from Lassen County, California, to British Columbia along the eastern fringe of the Cascade Mountains. The characteristic here is green and brown pigmentation on the under side hind wing. It should be remembered that this grades completely into *nevadensis*.

Argynnis callippe meadii for the Great Basin zone proper in which the yellow band is suffused with green scaling for the most part.

Some systematists may feel it desirable to extend further the analysis of the variations by applying names to even more restricted local populations. The author

does not think this necessary or desirable, but does consider it of importance that records of variations and distribution be published. The author believes the important part to be the variation and the biological significance of the variation, and this can be studied in any number of ways. For practical purposes of identification in a collection it is desirable that local populations be given names, but variation considered "unnamable" is often as important as, or more important than the latter and should receive its proper place in publications.

MATERIAL EXAMINED

The following list shows by region the approximate quantity of material examined by the author. Detailed listing of localities, numbers, etc., was considered to be too bulky for reproduction. San Francisco Bay area, except northern part, ± 700 individuals; Santa Lucia Mountains—Ventura area, ± 200; Ventura—Lower California area, ± 500; Sierra Madre—Greenhorn Mountains area, ± 400; southern to northern Sierra Nevada on west side, ± 60; southern to northern Sierra Nevada on east side, ± 400; southern Oregon and northern California, ± 300; north Coast Range area, ± 150; Steens Mountains, Oregon and eastern Nevada, ± 25; total of about 2,750, much of this being in the zones of intergradation and samples of specific, pertinent populations.

In addition to this, confirmatory data in the literature may be added. The author has personally collected in and become ecologically acquainted with populations in all the regions shown on the maps, with the exception of eastern Nevada. The area unshaded on the maps, except for those parts marked with a question mark, almost certainly harbor no population of this species, most of this area having been extensively investigated.

CONCLUSIONS

The author believes that the above data warrant the conclusion that geographic races or subspecies are units composed of populations having more or less similar

genes. The differences between races may be ascribed to different combinations of these genes existing in different populations which may be synthesized into any combination. Theoretically, any race may be formed with the proper genetic materials; in practice, this would be of considerable difficulty, since innumerable generations may be needed for selection to provide the proper "genetic environment" for the new gene combination. It is concluded that for the most part racial characters are either directly or indirectly adaptive (also the conclusion of Dice, 1940). It would not be fitting to formulate any definite conclusions on the origin of species from these data. However, authors seem to do so consistently on no more information. It should be noted that the term species is being used in much recent literature for units no larger than the smallest units suggested in this paper as possible races or subspecies. Finally, it may be concluded that subspecies or races are purely units of convenient classification.

SUMMARY

The geographical distribution and variation of *Argynnis callippe* in California and adjacent regions is described with a genetic viewpoint by which it is assumed that populations are different because they have different

genes.or different frequencies of genes. Maps are given which show that only two of six distinctive characters are always completely correlated, the distribution of the others bearing no definite relation to the others. Additional data are given to support the conclusion reached in a former paper that most characters distinguishing geographic races are adaptive in character, but that this adaptiveness is generally of a concealed nature.

NOTE. Since the above was in process of publication the following important information has been obtained. The gap in geographical distribution between the southern and northern parts of the north Coast Range has been partially filled by material from Comptche, Mendocino County, California. This population would be located on the map directly northwest of the northernmost denoted dot in the southern part of the north Coast Range (fig. 1, opposite the first bulge of the coast north of San Francisco Bay). Therefore, the range of the species extends closer to the coast in this region than was originally expected. In the characters shown by these specimens, which number twelve, the maps represent accurately the character combinations as hypothetically determined earlier. One specimen represents an intermediate condition between silvered and unsilvered spots (fig. 1), the farthest south in the Coast Range that such material is known. Verification of expected character combinations of this sort serves to show that one can predict population analyses nearly as accurately as one can predict results in the more experimental sciences. This material was examined and made known through the courtesy of C. D. Michener and L. P. Grey.

LITERATURE CITED[4]

Dice, L. R.
    1940. AM. NAT., 74: 212–221.
Hovanitz, W.
    1941. *Ecology*, 22: 259–284.

[4] The general evolutionary literature is not discussed in this paper because ready reference can be made to certain very recent and excellent general evolution treatises or to the author's paper cited where a bibliography is given.

THE EFFECT OF ZINC SULFATE UPON AN INBRED POPULATION OF DROSOPHILA MELANOGASTER[1,2]

DR. DONALD GREIFF

INTRODUCTION

THE present study was undertaken to determine the effect of zinc sulfate upon the population characteristics of a highly inbred strain of *Drosophila melanogaster*. Preliminary tests showed that the introduction of zinc sulfate into a synthetic medium caused a deleterious differential in the emergence of imagoes. The flies were inbred by brother and sister matings for eleven generations before the three following lines were established:

a. The control line; inbred flies raised upon the control medium.

b. The selected line; inbred flies raised from the twelfth to the thirty-second generation upon the test medium.

c. The selected-control line; inbred flies raised from the twelfth to the twenty-fifth generation upon the test medium and from the twenty-sixth to the thirty-second generation upon the control food.

Representative numbers of flies of the twelfth, sixteenth, twenty-fifth, twenty-sixth and thirty-second generations were tested for population characteristics as regards: fecundity;[3] productivity; time of imaginal emergence, the duration of time from mating to the emergence of adult offspring; weight of imagoes; inherent vitality.

[3] In order to avoid the confusion which has grown up in the literature, the terms "fecundity," "productivity" and "fertility" used in this report are those introduced by Hyde (1914b), who first brought to light the factors involved in the determination of fertility in this species. These were defined as follows: (1) Fecundity—the number of ova or sperm produced by an adult organism. (2) Productivity—the number of offspring arising from a single mating. (3) Fertility—the ratio between the number of eggs laid and the number of offspring issuing therefrom expressed in per cent.

MATERIALS AND METHODS

The ancestral flies employed in this investigation were obtained from Professor T. H. Morgan in December, 1919, by Dr. Raymond Pearl, of happy memory. They were of the Old Falmouth strain of *Drosophila melanogaster*, long inbred in Morgan's laboratory. Pearl and Parker (1922) further inbred this stock and from it raised their "107" line. This line was kept as a laboratory stock by pedigree breeding for several years and then carried along by mass brother and sister matings. The parental pair of the present study was made from a single brother and sister mating. This line was continued by brother and sister matings for eleven generations, from which the three lines of the present investigation were established. Thereafter the flies of the same generation and line, were grouped and randomly selected males and females were used as the parents of the succeeding generation.

The control medium used for the cultivation of the flies was a modification of the synthetic medium, S-101, developed by Pearl, Allen and Penniman (1926). Preliminary experiments with serial dilutions of various compounds showed that 10^{-3} gram of zinc sulfate per gram of synthetic medium acted as the best selective agent. Therefore, the test medium was formed by adding 0.10 gram of zinc sulfate to 99.90 grams of the control medium. The culture bottles were kept in a standard double-walled, biological incubator. The temperature was maintained at $25 \pm 0.3°$ C. by means of a bi-metallic thermoregulator. Humidity was accurately controlled by a saturated ammonium chloride (c.p.) solution as described by Obermiller (1924) and reported to maintain a humidity of 79.3 per cent. at 25° C. (International Critical Tables, 1926).

Flies, twelve hours old, were used in determining the duration of life without food. Each fly was put in an isolation tube and placed in the incubator. The technique used in handling the flies in the incubator was that de-

veloped by Powsner (1935): (1) clustering the isolation tubes as near as possible to the thermoregulator; (2) rotating isolation tubes randomly in the incubator; (3) keeping number of isolation tubes constant.

Fly weights were made upon a chainomatic balance. It was found that the greatest sensitivity of the balance occurred between .003 and .005 of a gram. The weights of five flies, 12 hours old, fell within this range. Therefore, to obtain the greatest accuracy in weight, the imagoes were weighed in groups of five. In order to obtain an exact measure of fecundity, it was necessary, after the total emergence of imagoes had been counted, to determine the number of undeveloped reproductive units. The medium within the culture bottle was melted and poured into several glass plates edged with rubber weatherstripping to act as a retaining wall. The culture bottle was washed with hot water to remove any eggs, larvae and pupae therein. The washings were added to the previously poured medium. After drying at room temperature, the plates were placed upon a modified bacterial colony counting apparatus and the unhatched eggs, dead larvae, non-emerged pupae enumerated. This method differs from that of Pearl (1932) and Alpatov (1932). Traumatizing of the various units is reduced to a minimum; 50 cc of medium is used instead of a few cc; the counts are made after a fairly long period of time and not, as the other techniques required, at the end of 24 to 72 hours. Thus this technique allows the cultures to approach the normal conditions of the laboratory.

EXPERIMENTAL DATA

The life spans of the several generations were as follows:

12th generation—February 1 to February 24.
16th generation—April 2 to April 27.
25th generation—July 25 to August 16.
26th generation—August 6 to August 28.
32nd generation—November 3 to November 28.

Fecundity

Table I gives the statistical constants for the fecundity of the generations tested. It is apparent that the introduction of zinc sulfate into the medium did not affect the fecundity of the females for several generations. Thus it

TABLE I

THE STATISTICAL CONSTANTS FOR THE FECUNDITY PER BOTTLE PER EIGHT MATED DAYS OF THE CONTROL, SELECTED AND SELECTED-CONTROL LINES

Generation	Control line		Selected line		Selected-control line	
	Mean (eggs laid in 8 days)	No. of bottles	Mean (eggs laid in 8 days)	No. of bottles	Mean (eggs laid in 8 days)	No. of bottles
F_{12}	370.62 ± 58.71	8	306.54 ± 33.58	11		
F_{16}	326.11 ± 59.01	9	313.81 ± 37.73	11		
F_{25}	328.78 ± 24.62	14	205.72 ± 37.91	11		
F_{30}	267.18 ± 16.40	16	142.21 ± 17.64	14	136.30 ± 21.01	13
F_{32}	191.93 ± 23.98	15	273.00 ± 9.92	14	199.37 ± 14.58	16

would appear that the mode of action of the agent used was different from the mode of action of the environmental factors employed by Delcourt and Guyénot (1911), Guyénot (1913a, 1913b, 1913c, 1913d), Richardson (1925), Hanson and Ferris (1929), Pearl (1932) and Alpatov (1932).

The technique of this research was such that egg laying over a limited time was measured. Thus the lowering of fecundity of the selected line admitted of three explanations: (1) the change in fecundity was due to a change in the germ plasm; (2) the change in fecundity was due to a loss of certain portions of the germ plasm; (3) the change in fecundity was due to a change in the physiological cycle of egg-laying and the greatest amount of eggs was laid after the eight-day period was passed. Hanson and Ferris (1929), Shapiro (1932), Alpatov (1932) have shown that in *D. melanogaster* the greatest number of eggs is laid in the first ten days. The foregoing was also shown for *D. pseudoobscura* by Dobzhansky (1935).

If the first alternative is correct, it would mean that simultaneous mutations took place in a large number of

flies in a fairly short time. To account for the rise in fecundity of the selected line and selected-control line between the 27th and 32nd generations would mean that a reversal to the initial status of the germ plasm occurred. The experience of many investigators with the rate of mutation makes the tremendous mutation rate necessary for the first alternative extremely remote.

It was found that the change in fertility of the 12th and 16th generations between the selected line and the control line was due to the loss of larvae in the selected line. If this loss of certain larvae meant the loss of germ plasm or of certain genes contained therein, the fecundity of the 16th generation would have been expected to be significantly less than the fecundity of the control line. However, this was not found to be the case and therefore the second alternative seems unlikely.

The fecundity of the control line and the selected line were not significantly different for the 12th and 16th generations. By the 25th generation the fecundity of the selected line was significantly less than that of the control line. It must be remembered that the amount of zinc sulfate imbibed by a single fly during its larval stage is extremely small. Therefore, it is possible that the zinc sulfate had no effect for the first several generations upon fecundity because the amount present was not great enough to influence the normal physiology of the female in this regard. However, by the 25th generation the concentration of zinc sulfate within the flies may have been such that it could act upon the function of egg-laying. By the 32nd generation, however, the flies could have developed a tolerance to the reagent present and returned to the normal cycle of egg-laying. In the selected-control line the return to the normal cycle may be accounted for by the dilution of the zinc sulfate present to such an extent that it no longer acted physiologically upon egg-laying. Thus it would seem that the change in fecundity was due to an upsetting of the normal cycle of egg-laying.

The downward trend exhibited by the control line was not significant. The fecundity ranged from 370.62 to

191.93 eggs per bottle per eight mated days. This is in keeping with the figures found by Hyde (1914a, 1914b, 1914c, 1914d), Shapiro (1932) and Alpatov (1932). Thus the results obtained by the technique used in this study are in keeping with the figures obtained directly by other investigators.

Productivity

Table II contains the statistical constants for the emergence of imagoes. There is no statistically significant difference in the emergence of male and female ima-

TABLE II

STATISTICAL CONSTANTS FOR IMAGINAL EMERGENCE OF THE CONTROL, SELECTED AND SELECTED-CONTROL LINES.

Generation	Control line		Selected line		Selected-control line	
	Mean (Imagoes per bottle)	No. of bottles	Mean (Imagoes per bottle)	No. of bottles	Mean (Imagoes per bottle)	No. of bottles
F_{12} ♂♂ + ♀♀	241.12 ± 35.21	8	156.72 ± 17.65	11		
♂♂	121.62 ± 19.03	8	77.90 ± 8.96	11		
♀♀	119.50 ± 16.51	8	78.81 ± 9.13	11		
F_{16} ♂♂ + ♀♀	171.88 ± 32.81	9	114.54 ± 14.57	11		
♂♂	83.55 ± 14.91	9	61.54 ± 6.61	11		
♀♀	88.33 ± 18.09	9	53.00 ± 8.49	11		
F_{25} ♂♂ + ♀♀	211.42 ± 18.89	14	114.27 ± 24.48	11		
♂♂	105.14 ± 8.61	14	59.63 ± 12.06	11		
♀♀	106.28 ± 10.46	14	54.63 ± 12.75	11		
F_{30} ♂♂ + ♀♀	177.37 ± 15.18	16	90.42 ± 13.85	14	90.15 ± 16.72	13
♂♂	87.00 ± 7.40	16	48.92 ± 7.52	14	44.69 ± 8.19	13
♀♀	90.37 ± 8.06	16	41.50 ± 6.71	14	45.46 ± 8.73	13
F_{32} ♂♂ + ♀♀	137.60 ± 24.28	15	204.00 ± 8.73	14	142.93 ± 16.07	16
♂♂	70.13 ± 12.97	15	103.53 ± 5.28	14	69.62 ± 8.03	16
♀♀	67.46 ± 11.50	15	100.00 ± 5.05	14	73.31 ± 8.40	16

goes of the several lines. This was contrary to the experiences of Hyde (1941a), Warren (1918) and Lawrence (1940). The foregoing investigators found that the ratio of emergence favored the females. The results obtained for this investigation were not unexpected, however, for the samples used for any one test were too small to show the existence of a sexual differential in emergence.

That a relationship exists between fecundity and productivity there can be little doubt. Castle, *et al.* (1906),

Moenkhaus (1911) and Hyde (1914c) demonstrated that close inbreeding did not diminish the productivity of *D. melanogaster* provided productive pairs were selected to continue the stock. This fact was again confirmed in the present investigation, although the control line exhibited a slight downward trend in productivity between the 12th and 32nd generations, and the selected line between the 12th and 26th generations. Since the samples used were small and the mean differences relatively small, it was felt that the means of the individual lines were randomly distributed.

The lower mean productivity of the 12th and 16th generations was due to the elmination of potential imagoes during the larval stage. The decreased productivity of the 25th and 26th generations was due to the lowering of the fecundity for those generations as pointed out in the preceding section. Thus the actual numbers of component units in the control, selected and selected-control lines were in the same ratio. With the return of the selected and selected-control lines to normal fecundity in the 32nd generation, the productivity of the lines also returned to normal. Therefore, it is apparent that the fertility of the flies was immediately affected by the presence of the zinc sulfate in the medium. By the 25th generation the fertility had returned to normal but the probable accumulation of the agent within the imago had lowered the productivity by lowering the fecundity. Thus the concentration of zinc sulfate necessary to disturb the fertility of the organism was small and adaptation was soon accomplished. After the tolerance to the zinc sulfate was established, however, it was great enough to buffer the effects of greater concentrations of the reagent.

Time of Imaginal Emergence

Table III gives the statistical constants for the time of emergence of the populations of the several lines following eight-day matings of the parental flies. The differences in the mean time of emergence of the males and

TABLE III

STATISTICAL CONSTANTS FOR THE TIME OF EMERGENCE (FOLLOWING EIGHT-DAY MATINGS) OF THE CONTROL, SELECTED AND SELECTED-CONTROL LINES

Generation	Control line		Selected line		Selected-control line	
	Mean (days)	No. of flies	Mean (days)	No. of flies	Mean (days)	No. of flies
F_{12} ♂♂ + ♀♀	14.61 ± .05	1934	14.98 ± .04	1724		
♂♂	14.64 ± .07	976	14.99 ± .06	857		
♀♀	14.55 ± .06	958	14.98 ± .06	867		
F_{16} ♂♂ + ♀♀	14.12 ± .11	1547	13.57 ± .07	1260		
♂♂	13.92 ± .16	752	13.66 ± .10	678		
♀♀	14.30 ± .16	795	13.46 ± .10	582		
F_{25} ♂♂ + ♀♀	12.41 ± .05	3185	12.24 ± .06	1257		
♂♂	12.49 ± .08	1473	12.21 + .08	656		
♀♀	12.34 ± .08	1712	12.27 ± .09	601		
F_{30} ♂♂ + ♀♀	12.97 ± .06	2835	12.76 ± .06	1266	11.60 ± .06	1175
♂♂	12.95 ± .08	1389	12.73 ± .08	685	11.68 ± .09	584
♀♀	12.99 ± .08	1446	12.80 ± .09	581	11.52 ± .08	591
F_{32} ♂♂ + ♀♀	13.53 ± .08	2067	12.75 ± .03	2879	13.00 ± .06	2288
♂♂	13.49 ± .11	1058	12.86 ± .05	1479	12.99 ± .09	1114
♀♀	13.60 ± .11	1009	12.64 ± .05	1400	13.02 ± .08	1174

females of the control line were not significant. This was also true for the selected line with the exception of the 32nd generation. The females of this generation emerged in a significantly shorter time than did the males. No significant difference was found in the selected-control line.

The combined sexes, males separately, and females separately of the control line seemed to have a cyclic trend. The time of emergence was greater during the winter months than during the summer months. The longest time of emergence for this series was the 12th generation, and the shortest time of emergence was at the 25th generation. It is a well-known fact that the physiological processes of an organism vary greatly during the course of a year. This is especially noteworthy when the organism, with a cycle in its natural habitat, is brought into the laboratory. In this regard it has often been found that even when the environmental conditions are controlled for experimental purposes, the seasonal variation often persists. Thus Schneider (1940b) working with *Tribolium confusum* reported that the length of larval period was longest in October and November,

shortest in June and July. He concluded that the spring and summer broods developed faster than the fall and early winter broods.

Therefore, it is not at all surprising that the mean time of emergence in this investigation displayed a cyclic trend. However, several other facts must be considered. Bliss (1926) found that the higher the temperature, the shorter the time of prepupal development in the males and females of *Drosophila melanogaster*. Bonnier (1926) using a sex-linked mutant yellow stock reported that the mean time of emergence from eggs to imagoes at 25° C was 232.21 hours for the males and 227.98 for the females. At 30° C the developmental times were 187.63 hours for the males and 178.10 hours for the females. Alpatov (1930) working with the wild-type fly reported that the time from egg-laying to pupation required 93.16 hours at 28° C and 200.86 hours at 18.2° C. Thus the results obtained for the present work would have been expected if the temperature at which the cultures were kept had varied simultaneously with the external temperature. However, as was pointed out earlier, the environment surrounding the flies was kept uniform from generation to generation. But it must be recognized that other but less obvious meteorological phenomenon accompany changes in temperature. Thus the cycle may be due to one of two factors: (1) the cycle was due to uncontrolled manifestations present in the atmosphere surrounding the incubators; or (2) the cycle was due to the genetical make-up of the fly. The cycle may also be the resultant of the interaction of both of the foregoing factors.

The mean time of emergence of the selected line for the sexes combined, males separately, and females separately exhibited the same trend as the control line from the 12th to the 26th generation. Instead of continuing its upward swing, as was found for the control line, there was a leveling off and the mean of the 26th and 32nd generations were not significantly different. Therefore, it would seem that the accumulation of the agent in the selected flies

again reached a threshold at which point it caused a change in the normal physiology of the fly. The trend of the selected-control line was like that of the control line for the generations tested. The mean time of emergence of the 32nd generation was significantly higher than the mean of the 26th generation for the sexes combined, for males and females separately. The females of the selected line were also found to be more rapidly returning to the control position than the males, following the removal of the deleterious agent. Thus it would appear (1) that the males are more susceptible to the effect of the agent or (2) a change has occurred in the germ plasm through the use of the agent.

Weight of the Imago

Table IV gives the statistical constants of the weights of five-fly groups. In keeping with other observations on insects, Eigenbrodt (1925) and Schneider (1940a, 1940b,

TABLE IV

STATISTICAL CONSTANTS FOR WEIGHTS OF IMAGOES OF THE CONTROL, SELECTED
AND SELECTED-CONTROL LINES (IN TERMS OF GROUPS OF FIVE FLIES)

Generation	Control line Mean (gm.)	No. of groups	Selected line Mean (gm.)	No. of groups	Selected-control line Mean (gm.)	No. of groups
F_{12} ♂♂ + ♀♀	.00461 ± .00011	49	.00423 ± .00014	49		
♂♂	.00377 ± .00010	19	.00357 ± .00012	27		
♀♀	.00514 ± .00009	30	.00503 ± .00016	22		
F_{10} ♂♂ + ♀♀	.00414 ± .00008	57	.00445 ± .00009	53		
♂♂	.00367 ± .00007	27	.00396 ± .00008	29		
♀♀	.00456 ± .00010	30	.00505 ± .00008	24		
F_{25} ♂♂ + ♀♀	.00449 ± .00006	101	.00454 ± .00008	69		
♂♂	.00392 ± .00003	51	.00395 ± .00002	37		
♀♀	.00508 ± .00004	50	.00522 ± .00005	32		
F_{26} ♂♂ + ♀♀	.00446 ± .00007	88	.00439 ± .00007	87	.00448 ± .00009	81
♂♂	.00396 ± .00003	44	.00378 ± .00004	45	.00367 ± .00002	39
♀♀	.00528 ± .00005	44	.00504 ± .00005	42	.00522 ± .00005	42
F_{32} ♂♂ + ♀♀	.00419 ± .00009	55	.00433 ± .00006	85	.00425 ± .00012	56
♂♂	.00358 ± .00007	27	.00379 ± .00004	42	.00353 ± .00007	30
♀♀	.00477 ± .00009	28	.00487 ± .00005	43	.00509 ± .00013	26

1940c, 1940d, and 1940e) found that the females were significantly heavier than the males. This held for the several lines and all the generations of this investigation

tested. The control line males seemed to have a cyclic
trend in regards to weight. The peak occurred at the
25th and 26th generations, the troughs at the 16th and
32nd generations. Thus the males went through a single
cycle in a ten-month period. The control line females
also appeared to possess a cyclic trend in weight. The
peaks occurred at the 12th and 26th generations, the
troughs at the 16th and 32nd generations, Thus the
females went through one and one-half cycles in a ten-
month period.

The presence of zinc sulfate disturbed the normal
cycle in both the males and females of the selected line.
The mean weights of the males tended to vary little in the
later generations tested, although in the earlier genera-
tions the cycle was much like that for the control line
males. Thus it would seem that after a certain concen-
tration of zinc sulfate was present within the fly a change
in its physiology occurred. This is further shown by the
fact that the removal of the reagent from the medium
resulted in the mean weights returning to the control
position. The physiology of the females was immediately
affected by the zinc sulfate. The effect was such that they
showed little variation in mean weight from the 12th to
the 26th generation. By the 32nd generation, however,
an adaptation seems to have occurred and the mean
weight was not significantly different from that of the
control. The removal of the environmental agent did
not change the physiology of the females. From the fore-
going it would appear that the zinc sulfate acted dif-
ferentially on the sexes. The males tended to preserve
their cycle but continued breeding on the test medium
finally upset this tendency. The females, on the other
hand, were affected immediately but with continued breed-
ing tended to return to the normal (control) cycle.

*Inherent Vitality of Imago Measured by the Dura-
tion of Life in the Absence of Food*

Table V gives the statistical constants for the inherent
vitality of the newly emerged imago. The mean duration

of life of the females of the control line of this investigation were significantly greater than the mean of the males in each generation tested. This conforms with the findings of Pearl and Parker (1924), who state: ''The normal relation between the sexes in respect of mean duration of life (females longer-lived than males) observed under full feeding, is preserved under conditions of complete starvation.'' Greiff (1940) found that the males of the wild-type fly lived significantly longer than the females under a condition of complete starvation. Hyde (1913)

TABLE V

STATISTICAL CONSTANTS FOR THE INHERENT VITALITY OF THE CONTROL, SELECTED AND SELECTED-CONTROL LINES

Generation	Control line		Selected line		Selected-control line	
	Mean inherent vitality (hours)	No. of flies	Mean inherent vitality (hours)	No. of flies	Mean inherent vitality (hours)	No. of flies
F_{12} ♂♂ + ♀♀	44.55 ± .53	381	46.92 ± .54	334		
♂♂	43.03 ± .77	198	46.10 ± .77	184		
♀♀	46.19 ± .71	183	47.92 ± .73	150		
F_{16} ♂♂ + ♀♀	38.10 ± .54	425	38.58 ± .59	418		
♂♂	34.15 ± .81	205	35.26 ± .80	221		
♀♀	41.78 ± .64	220	42.30 ± .77	197		
F_{25} ♂♂ + ♀♀	26.03 ± .48	345	23.70 ± .49	246		
♂♂	23.82 ± .72	169	22.33 ± .66	144		
♀♀	28.15 ± .65	176	25.64 ± .68	102		
F_{30} ♂♂ + ♀♀	31.82 ± .48	290	32.87 ± .59	242	30.94 ± .47	217
♂♂	30.08 ± .61	148	30.29 ± .76	121	29.77 ± .62	105
♀♀	33.63 ± .73	142	35.45 ± .86	121	32.03 ± .69	112
F_{32} ♂♂ + ♀♀	28.18 ± .60	245	26.56 ± .64	300	29.16 ± .48	386
♂♂	26.23 ± .92	121	24.71 ± 1.01	143	26.58 ± .74	197
♀♀	20.09 ± .75	124	28.24 ± .77	157	31.84 ± .56	189

found the foregoing to be the case with fed imagoes. It is not unlikely that the differences in physiological response were due to the fact that Hyde and Greiff used different strains of flies from those used by Pearl and Parker. This is probable in light of the fact that the flies used in the investigation of Pearl and Parker were the ancestral parents of the flies of this research.

The females of the selected line were longer lived than the males. The differences were significant for every generation excepting the 12th. The differences in mean duration of life of the selected-control line females were

significantly greater than the males. Thus it would seem that the agent used was able to upset the normal relationship of males to females for this strain. However, an adaptation occurred after the 12th generation and the physiology of the fly returned to its normal inherent vitality.

The mean duration of life without food appeared to possess a cyclic trend. It was found, however, that the amplitude of the waves of the cycle was dampened by inbreeding. The control and selected lines behaved similarly. The peaks were at the 12th and 26th generations, the troughs at the 25th and 32nd generations. The zinc sulfate acted as a stimulant on the 12th generation selected males, but by the 16th generation the difference was not significant. The removal of the zinc sulfate did not alter the mean duration of life without food. Thus it would seem that the reagent had little influence on inherent vitality.

INCIDENTAL OBSERVATIONS

The following observations were noted during the course of this study:

(1) The selected line flies required approximately twice the amount of ether and about twice as long to anesthetize as the control line flies.

(2) Anesthetized selected line flies displayed a tetany of the wing muscles.

(3) The control medium possessing control flies was firm for about 20 days; the test medium possessing selected line flies rapidly became loose at the end of three days.

The foregoing also held for the control medium having selected line flies.

SUMMARY

(1) Three lines of inbred populations of *Drosophila melanogaster* were established: (a) the control line composed of flies raised on synthetic medium; (b) the selected line raised on synthetic medium plus zinc sulfate; and (c) the selected-control line raised for several genera-

tions on the test medium and brought back to the control medium.

(2) Representative numbers of flies of several generations were tested for their population characteristics, namely: fecundity, productivity, time of emergence, weight of imago, and inherent vitality.

(3) Preliminary experiments showed the 10^{-3} gram of zinc sulfate per gram of synthetic medium acted as the best selective agent of the several concentrations tried.

(4) The fecundity of the control line was not affected by inbreeding.

(5) The fecundity of the selected line was lowered by the presence of the zinc sulfate but in later generations an adaptation occurred.

(6) The zinc sulfate seemed to delay the laying of the eggs.

(7) The productivity of the flies was lowered by the zinc sulfate, but in later generations this returned to normal, seemingly through adaptation.

(8) The time of emergence from mating to adult offspring showed a cyclic trend.

(9) The control line males and females exhibited a cyclic trend in weight; the males went through a single cycle in a ten month period, while the females one and one-half cycles in the same period.

(10) The zinc sulfate disturbed the weight cycle which was immediately apparent in the males but delayed in the females.

(11) An adaptation occurred in the females in later generations and the cycle for weight returned to normal.

(12) It was pointed out that the various results reported in the literature on inherent vitality was probably due to the use of different strains of *D. melanogaster*.

(13) Duration of life without food appeared to possess a cyclic trend with the amplitude of the waves dampened by inbreeding.

(14) The zinc sulfate had little effect upon inherent vitality as measured by duration of life without food.

Conclusions

Zinc sulfate introduced into the culture medium reduced the productivity and fecundity of an inbred population of *Drosophila melanogaster*. This reduction was evident for several generations as measured by the control. The effect, however, was not permanent as these functions returned to normal even in the presence of this agent on further inbreeding. Apparently these two physiological characteristics became adapted to the new environment.

There also occurred a sexual differentiation in weight, as the normal cycle was disturbed for both sexes. Continued inbreeding restored the cycle in the females but not in the males at the end of the 32nd generation.

The time of emergence of the imago gave a cyclic trend that was disturbed by the zinc sulfate. This was not restored on continued inbreeding up to the 32nd generation.

The duration of life of the imago in the absence of food possessed a cyclic trend that was not affected by the zinc sulfate.

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THE USELESSNESS OF THE SPINDLE FIBERS FOR MOVING THE CHROMOSOMES

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INTRODUCTION

In a previous paper (Piza, 1939) it was pointed out that the chromosomes of *Tityus bahiensis* are inserted at the spindle very prematurely, that is, at a time long before the metaphase of the first spermatocyte division. If the chromosomes were provided as usually with a single localized insertion region, then this peculiarity wouldn't be noticed or at least would draw no special attention from the investigator. But, since the chromosomes, as was shown in several papers (Piza, 1939, 1939a, 1940, 1941), are provided with a point of attachment at each extremity, the fact referred to above acquires an enormous significance, permitting the formation of a more or less exact opinion concerning the part played by the spindle fibers in moving the chromosomes.

The outstanding question is simply to decide whether or not the fibrillar elements of the spindle connected with the chromosomes have any influence upon the latter, pulling them toward the poles or merely guiding them while some unknown forces act on their separation.

The theories attempting to explain the movement of the chromosomes are many, and were recently summarized by Schrader (1940). Among these, one—the traction theory or "Zugfaserntheorie" of the German authors—had assumed that the fibers, anchored at the centers and attached at the other end to the chromosomes, could pull the chromosomes to the poles. This theory was criticized mainly because of the fact that the fibers, as they become shorter, never seem to become correspondingly thicker. Thus, it seems clear that the reduction in length

of the fibers can not be attributed to a true contraction effect.

Based on his studies on Tityus, Piza (1939a) again came to describe the filaments of the spindle as effective agents in the movement of the chromosomes, considering them as a material support which the chromosomes have to absorb in order to get to the poles. The mechanism proposed, suggested by a decrease in consistency responsible for the bending of the chromosomes toward the poles, due to the absorption of the spindle material by the point of attachment, seemed to him accountable for the progressive reduction in length of the fibers not accompanied by any increase in diameter.

It is a well-established fact that the capacity of the chromosomes to perform regular anaphase movements is directly associated with their power in producing spindle fibers and that the akinetic chromosomes lose concomitantly both the faculty of moving normally as well as that of originating spindle fibers. Whether or not these faculties depend upon one another is not known. However, the fact that the chromosomes of Tityus are attached at the spindle long before metaphase is reached does modify completely any point of view which considers the fibers as mechanical agents in the anaphase movements, even in the passive manner proposed by Piza.

THE FACTS

A complete account of meiosis in Tityus will be given in another paper. In the present one only some very remarkable features of the first spermatocyte division will be reported.

When the zygotene stage is finished the nucleus of the spermatocyte appears occupied by thin threads of irregular chromomeric outline each of which is formed by two strands intimately united along their whole length. In its complicated way within the nucleus each thread describes a wide spiral whose segments are separated by very narrow turns. From this stage till metaphase the

chromosomes pass through a continuous process of length reduction, on twisting and surface uniformization, showing more and more clearly their double parallel nature. The process of spindle formation could not be followed. But the spindle is completely set up long before the chromosomes acquire their ultimate metaphase shape. At that time the bivalents, formed by two parallel
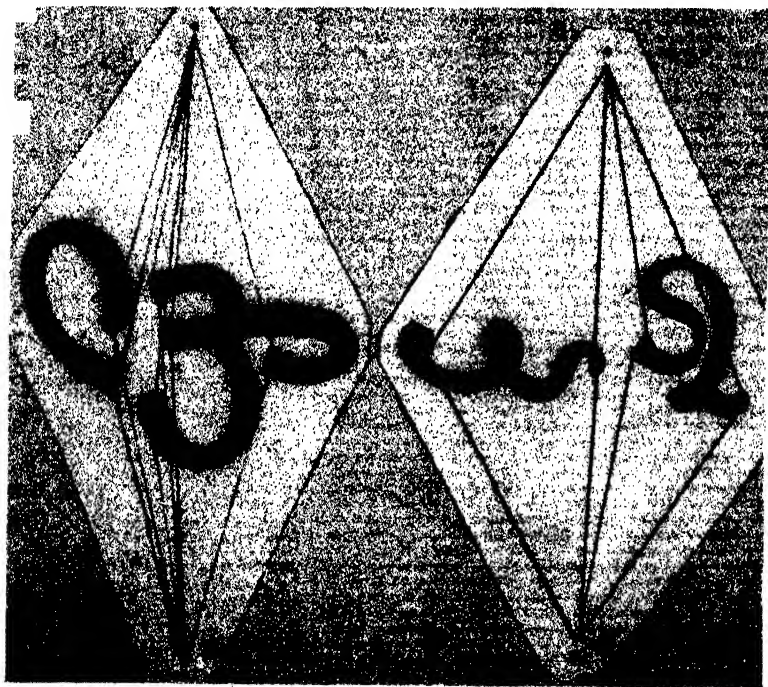
FIG. 1.   Tityus chromosomes attached at the spindle long before metaphase of the first spermatocyte division. These chromosomes are far from reaching their ultimate rod-shaped form. FIG. 2.   Long spiralized chromosomes of the first spermatocyte division of Tityus already attached at the spindle. In spite of having both extremities connected with the poles by means of spindle fibers, these chromosomes can move and rotate freely in order to assume their ultimate metaphase shape.

threads, are still more or less twisted, have their extremities out of the plane of the equator and are bent in the most varied manner. Moreover, they are already attached at the spindle by their two ends (Figs. 1 and 2).

Finally, becoming shorter the chromosomes finish by being divested of their last spiral twists and by orienting themselves in the equatorial plane, in such a manner that each component of a pair has both extremities directed toward the same pole (Fig 3). This orientation is sometimes accomplished before the chromosomes have reached their final length. The metaphase rod-shaped chromosomes may appear straight or bent. In lateral view many
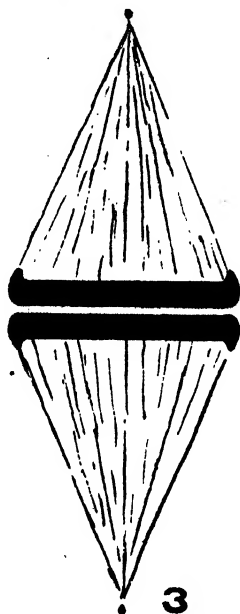
FIG. 3. Typical metaphase I chromosome of Tityus.

very thin fibrillae can be detected along the entire polar side of the chromosomes (Fig. 3). In discussing the significance of the spindle fibers, only those attached at the extremities of the chromosomes will be taken into consideration. In top view the paired chromosomes seem to be already divided, since something like a split gives to the bivalents a quadripartite aspect. However, considering that this particular feature can be analyzed with certainty in no other situation, it has been differently interpreted by Piza (1939a, footnote 15). Some prometaphase figures suggest that the extremities of the

chromosomes anticipate their body in reaching the equatorial plane (Figs. 1 and 2). The chromosomes are held together by a mutual attraction, since no chiasmata can be detected at any stage of meiosis.

DISCUSSION

Since fiber formation precedes anaphase movements and spindle fibers disappear as the chromosomes advance toward the poles, it seems probable that chromosome movements are dependent on the fibers produced by them before they begin to separate. Moreover, as was shown specially by Carlson (1938, 1938a, 1940) in grasshopper neuroblast, akinetic fragments of chromosomes, which do not develop spindle fibers, are also devoid of the capacity of regular orientation, being unable to establish any normal connection with the spindle. On the other hand, all movements prior to separation and even the orientation movements take place without spindle fibers. Thus Schrader (1941) has shown recently that the chromosomes of *Anisolabis maritima* move inside the nucleus toward the part of its wall which corresponds externally to the position of the centrosomes (as happens also in other species in the so-called bouquet stage) and, as the centrosomes separate from each other, the chromosomes, forming two groups, accompany them in their way to the poles.

The instrumentality of the spindle fibers in moving apart the chromosomes appears therefore unnecessary. Indeed, chromosomes which move and orient themselves independently of the fibers do not need to be attached to any special kind of fibrillar elements in order to get to the poles. The behavior of Tityus chromosomes comes in support of such a view. Actually, in this scorpion the spindle is well established when the chromosomes are still formed of long threads intimately united and twisted (Fig. 1). From that time on the chromosomes are seen to be connected with the poles by filaments attached at their extremities. The production of the fibers precedes

not only the occupation of the equatorial plane by the chromosomes but also the orientation of their ends with regard to the poles. When the extremities of the chromosomes are about to take a place in the plane of the equator they are already connected with both poles. Notwithstanding this, in their movement, as they go farther and farther from one pole they approach more and more the opposite one, without altering in any way their fibrillar connection with both. In addition to this, the bivalents are frequently bent in the most varied manner, so that, to assume the characteristic rod-shaped metaphase form they have to perform unwinding movements, which they do easily as if no fibers were present at their ends. The same is observed during orientation. In order to avoid bridge formation, that is, to secure a perfect separation, it is necessary that the chromosomes become completely untwisted so that both extremities of one partner can go to one pole while the extremities of the other go to the opposite one. In doing this the points of insertion have to reverse repeatedly their connection with the poles, looking now to the one then to the other. Finally, to pass from some intricate prophase situations to the very simple metaphase one we are forced to assume that the fibers can cross through one another or even through the body of the chromosomes without being broken or deranged. All this indicates that the spindle fibers do not have any influence in moving the chromosomes. Unfortunately, the present investigation furnishes no information concerning the nature of the spindle fiber itself. But, whatever may be the ultimate nature of the fibers they are to be considered as a component of the spindle continuously produced between the points of insertion and the poles whatever may be the relative situation of these points. They are the inevitable consequence of some unknown forces developed between the poles and each individual point of attachment, as the luminous bundle which crosses the space of a dark room is the consequence of the dust which fills the room and of an orifice

in the wall giving entrance to the light from the outside. It seems to me that the following assumption may point the way to the solution of this important problem: The fibers stretched from the insertion region of the chromosomes to the poles may well be considered as the result of a very feeble coagulation process due probably to something like an electrical force between these points, which determines the formation of something like a colloidal fibril exactly in the line connecting the two points. If the kinetochore changes its position with regard to the pole, filaments are formed in every new line while the colloidal material entering in the constitution of the preceding ones goes again into the sol state.

Returning to the facts, I do not know how far the results presented here can be generalized. However, there are in the literature at least two cases which are in full agreement with my findings in Tityus. One of these is the case of Protenor, the other the case of Sciara.

In Protenor, as was pointed out by Schrader (1935), the X-chromosome behaves differently in the two meiotic divisions of the spermatocyte. In the first division the X-bivalent lies flatly on the equatorial plane and after separation both chromosomes move parallel toward the poles. In the second division the X-chromosome, which does not divide, takes in metaphase a position perpendicular to the plane of the equator and without modifying that position goes indifferently to one or other pole. What is specially to be emphasized here is the fact that in moving toward one of the poles the X-chromosome of the second spermatocyte division of Protenor maintains a fibrillar connection with both, so that, as it moves, the spindle fibers become progressively shorter forwardly and correspondingly longer backwardly. The chromosome, therefore, moves freely as if no fibers were attached at its extremities.

The Sciara case studied by Metz and collaborators (1926) is equally demonstrative. In the maturation division of the spermatocytes of this fly the ten chromosomes

of the diploid set, without conjugation, put themselves individually in fibrillar connection with a single pole. Then, while six of the chromosomes move toward this pole as in an ordinary mitosis the other four go away from it, to form in the antipolar region a bud in which they are eliminated. What is singularly interesting in Sciara case is the backwards movement of the elimination chromosomes. In fact, these chromosomes, as they are going away from the pole, maintain their point of attachment constantly connected with it by means of spindle fibers. This extraordinary occurrence affords a strong support to the view developed in the present paper.

The very recent investigation of Pease (1941) on pressure effects upon the spindle figure and chromosome movement brought forward valuable experimental data for a more adequate interpretation of the mechanics of mitosis. Subjecting Urechis eggs to different hydrostatic pressures during various stages of the first mitotic cycle, Pease came to the following important results:

(1) Destruction of visible spindle begins with a pressure less than 2,000 lbs/in², being complete with a pressure of 3,000 pounds. By 3,000 pounds' pressure no trace of fiber structure is left, but the spindle area remains demarked by granulations slightly orientated longitudinally. With increasing pressures this granulated area begins in its turn to disappear, being entirely absent when the pressure reaches 10,000 pounds.

(2) Chromosome movements are perfectly normal under 1,000 pounds' pressure, are more and more slowed by pressures higher than 2,000 pounds, and more or less completely blocked by 6,000 pounds' pressure.

(3) Under pressures exceeding 2,000 pounds early anaphase chromosomes aggregate in fluid "vesicles" which move as units. While moving apart the vesicles generally remain connected by bridges.

(4) After the release of pressure new functional half-spindles are formed which pick up chromosomes at all mitotic stages from metaphase on.

As it was pointed out above, the spindle fibers are to
. be regarded as an inevitable consequence of a coagulation
process developed between the point of attachment of the
chromosomes and the poles. The immediate cause of the
coagulation which creates the fibers connecting chromo-
somes and poles is at the present state of our knowledge
entirely unknown. That the spindle fibers do not repre-
sent any particular structure of the dividing cell which
cooperates effectively in the movement of the chromo-
somes was emphasized by Gurwitsch (1926). In the
opinion of that writer the achromatic figure is a resultant
devoid of signification of the forces working in the bipolar
field in which it is formed. The spindle then would
be the morphological expression of the forces of a
bipolarity set up relatively early in the dividing cell,
which in the favorable situations may be recognized
shortly after the cell enters into mitotic activities.
Though Gurwitsch's conception, according to which the
chromosomes would be elements arisen *de novo* as a con-
sequence of gelatinization of substances produced within
preestablished lines (Bahnen), can not be accepted due to
many reasons not discussed here, there is nothing against
the idea developed by him that the mitosis may be the
result of the establishment of a field of bipolarized forces
in the cell. It is true that Gurwitsch left untouched the
fundamental question of the primary origin of the bi-
polarity. But, as soon as this bipolarity is set up, all ele-
ments of the cell under the action of the working forces
move following the vectors representing those forces. No
element of the cell is charged with any special activity.
Even the chromosomes are considered as members of a
dynamical system moving as a whole. Since no reference
is made to the probable kinetic influence of the central
bodies upon the chromosomes as well as to the cases in
which chromosomes move independently as if they be-
longed to different minor systems, the theoretical con-
siderations of Gurwitsch offer no ground for a more
detailed analysis.

Hughes-Schrader and Ris' (1941) recent investigation has shown that x-ray-induced fragments of the Steatococcus chromosomes behave mitotically like unbroken chromosomes, each fragment being typically oriented at metaphase, producing its own half spindle, and the parallel halves disjoining without lagging at anaphase, through many cell generations. Though rarely, some cases have been recorded in course of Steatococcus investigation, in which chromosome fragments vesiculate at metaphase or anaphase or lag on the spindle without producing spindle fibers. Vesiculated fragments, however, frequently showed autonomous anaphasic separation.

Now, returning to Pease's results we will see that chromosomes can still move at a time when the spindle fibers have disappeared completely. But, at that time the spindle substance is still present in the spindle area as more or less dispersed granules. Whether or not the total suppression of chromosome movement coincides exactly with total disappearance of spindle fiber substance is not clear. What is clearly shown is that the rate of chromosome movement is normal when the spindle fibers are equally normal and becomes more and more slow till complete cessation as the fibers are entering into a more and more pronounced disorganization. With the suppression of the hydrostatic pressure chromosome movement and spindle fibers come again to normality.

Altogether the facts discussed above point to the conclusion that the spindle fibers are nothing else than the optical expression of a physiological activity of the chromosomes, that is, of their power of responding with coordinated movements to the action of the forces operating during mitosis. Or, in other words, the spindle fibers are the consequence of the chromosomes' capacity of moving. Chromosomes or chromosome fragments (Steatococcus case) which move, determine spindle fibers formation, while chromosomes (Vivipara case reported by Pollister, 1939) or chromosome fragments (grasshopper

neuroblast) which have lost the faculty of moving regularly, are unable to produce spindle fibers. In Pease's experiments the chromosomes physiologically altered by increasing pressures lose progressively their reactivity to the moving stimulus from the poles, which in its turn became probably weaker, so that, when the chromosomes reach inactivity the power which determines the formation of a fibrillar gel connecting them with the poles being smaller than the opposite solation power of the hydrostatic pressure, no spindle fibers can be produced.

The point of attachment. A fact well established by the students of the cell is that the chromosomes are universally attached at the spindle by a single point. This point of attachment has received many different names among which primary constriction and kinetochore are the most common. In some cases the presence of a minute deeply staining granule has been noted in the insertion region of the chromosomes. When this granule is absent and the primary constriction obscure the position of the point of attachment is indicated in the long chromosomes by a pronounced repulsion which marks the beginning of their separation, as well as by the shape of the anaphase chromosomes, which, being bent exactly at the point of insertion, form an angle with equal or unequal sides according to the localization of that point.

In addition to the chromosomes of Ascaris germinal lineage whose compound nature was established some time ago, there are in the literature a number of cases in which the behavior of the chromosomes in anaphase suggests new methods of spindle attachment. Among these I wish to discuss here only the three which were considered by the respective authors as truly distinct from the typical cases. These are the case of Tityus (Piza, 1939, 1939a, 1940, 1941), the case of Steatococcus (Hughes-Schrader and Ris, 1941) and the case of the X-chromosome of Protenor (Schrader, 1935).

Tityus case. Tityus chromosomes are considered as undoubtedly provided with a spindle attachment at each

extremity. This is proved (a) by the strong repulsion at both ends clearly observed at metaphase and anaphase of the first meiotic division of the spermatocytes; (b) by anaphase movement in which the chromosomes, having the extremities turned toward the poles, begin to separate in perfect parallelism but assume later an arch-shaped form; (c) by the repulsion at a single extremity in spontaneous fragments and (d) by the similar behavior of the chromosomes at the second division of the spermatocytes.

At the metaphase of the spermatocytes I in which the existence of two kinetochores can be observed in the clearest way, the chromosomes, besides the fibers connecting the extremities with the poles, show in the triangular space formed by these fibers and their body a continuous pellicle of striated appearance. Since the beginning of my work on the scorpion I considered this structure as a pellicle and not as row of independent fibers because in oblique view of the chromosomes the fibrillar elements seem to belong to something like a very delicate film. This pellicle (if my interpretation is correct) is individual, that is, each chromosome possesses its own, so that each half spindle is seen to comprise three independent pellicles joined by their apices at the centrosomes.

The spindle, generally speaking, presents in fixed material a fibrillar aspect due most probably to a gelification of substances in a bipolarized field of forces. (See for discussion Piza, 1939a.) In this report, only the fibers attached at the kinetochores and due to an effect proceeding from them have been considered as true spindle fibers.

In Tityus only the fibers derived from the extremities of the chromosomes have a predetermined and constant point of origin. All other fibrillar elements seen along the body of the chromosomes seem to have neither a determined point of insertion nor any constancy in number. Consequently, I did not have any solid ground for interpreting these fibrillae other than as a secondary product of the coagulation of the material enclosed in the triangular field occupied by them.

The idea that Tityus chromosomes may be provided with a linear series of kinetochores as the compound chromosomes of Ascaris or with a diffuse spindle attachment as suggested by Schrader (1935) in the case of Protenor and by Hughes-Schrader and Ris (1941) in Steatococcus is contradicted by all facts presented above in favor of the existence of two terminal kinetochores.

With so many concrete arguments in support of the existence of a kinetochore at each extremity, the assumption that other points of attachment exist, suggested only by the presence of fibrillar elements along the entire length of the chromosomes, would be unnecessary since these elements could be interpreted as due to the coagulation of the material contained within the fibers of the extremities and the body of the chromosomes.

Piza (1939a) has considered the spindle as being homogeneous. The fibers connecting the chromosomes with the poles would result from the coagulating activity of an enzyme put out by the points of insertion. Any other fibers which would appear there by means of natural or artificial (due to fixation) processes would follow the lines of force of the spindle and could adhere to the body of the chromosomes laid across their way. These fibers would differ from the terminal ones in having no fixed point of insertion. The enzyme to which the gelification of the spindle was attributed would determine the formation of a fiber following exactly the line of force passing by the point it came from. In this manner the fact that the points of insertion are always attached at the end of a fiber would be intelligible. The interpretation given in the present paper, however, seems to be more adequate.

Steatococcus case. In spindle structure and anaphase chromosome movements the Mexican coccid *Steatococcus tuberculatus* recently studied by Hughes-Schrader and Ris (1941) presents a striking similarity to, *Tityus bahiensis.* Thus, in metaphase the chromosomes of Steatococcus lie with their entire body on the plane of the equator; at anaphase the chromosomes separate in per-

fect parallelism showing besides the fibers attached at their extremities many others distributed along the whole length of their polar face; in the end of anaphase the chromosome extremities bend toward the poles.

Steatococcus chromosomes, however, differ from Tityus chromosomes in the complete absence of the end repulsion, in consequence of which they maintain their metaphase parallelism until the end of anaphase, when they bend their ends toward the corresponding pole. The absence of any localized repulsion making impossible the detection of the position of the kinetochores, led the authors to the assumption of diffuse attachment, that is, of individual half spindles extending from one extremity to the other of the polar side of the chromosomes. Nevertheless, there is a particularity in the behavior of the Steatococcus chromosomes which permits a quite different interpretation. I mean the bending of the extremities of the chromosomes toward the poles in the end of anaphase. The authors did not pay any special attention to this fact, considering it as the effect of pressure on the whole chromosome from the narrowing space of the polar cone.

In the opinion of the present writer, the chromosomes of Steatococcus are, like Tityus chromosomes, provided with a point of attachment at each extremity. But, being relatively thicker and probably more rigid than in Tityus, the chromosomes of Steatococcus oppose resistance against the bending effect concentrated in both extremities, which, in this manner, can not be distinguished from the more or less generalized effect tending to separate the halves of the chromosomes. The resistance of Steatococcus chromosomes against bending is increased by the fact that each chromosome is constituted by two parallel chromatids. In the end of anaphase, however, due to a decrease in the viscosity of the chromosomes they become concave toward the corresponding pole, thus revealing the greater kinetic power of the extremities.

The explanation of Hughes-Schrader and Ris for the bending of the chromosomes seems to me inconsistent because if we admit the presence of a diffuse kinetochore, then we are forced to assume that the chromosomes, compelled to become bent when they reach the narrower parts of the spindle, would do so exactly in an opposite direction, that is, with the convexity facing the poles. If they really penetrate the polar zones of the spindle with the ends directed forwardly it is because the kinetic centers are localized at the ends.

As was referred to above, chromosome fragments of Steatococcus obtained by means of x-ray treatment behaving themselves like unbroken chromosomes were taken as proof for the reality of the diffuse kinetochore. This question will be discussed below.

Protenor case. The case of the X-chromsome of Protenor and of all other long chromosomes which behave in the same way may be joined to Tityus and Steatococcus cases. What makes the chromosomes of Tityus and Steatococcus take in the equatorial plane such a position that the components of each pair or double element look toward opposite poles is the existence of two kinetochores (undivided as in Tityus or already divided as in Steatococcus) at their ends. Mitosis studied in the embryos of Tityus as well as of Steatococcus has shown that in metaphase the separation of the chromosomes is already complete. There being two kinetochores directed toward opposite poles in each extremity of the double metaphase chromosomes, they separate parallel to each other.

The X-chromosome of Protenor, in the first division of the spermatocytes, which is equational, is evidently in an identical situation. The fibers attached along the entire length of the polar face of the X-chromosomes and their parallelism at anaphase led Schrader (1935) to propose for the first time a diffuse mode of spindle attachment. However, the behavior of this chromosome passing undivided to one of the poles in the second mitosis was left without explanation, since it moves parallel to the

spindle axis and maintains the extremities connected with the corresponding pole by means of a delicate fiber. But it seems to me that this peculiarity may be accounted for by the presence of a kinetochore at each end of the chromosome. Really, the kinetochores being single and localized just at the ends as Schrader's figures suggest, the influence the poles exert on them sooner or later would force the chromosome to take a position more or less parallel to the axis of the spindle.

A New Theory of Meiosis

Taking all these facts into consideration I will try in the following paragraphs to develop a general theory of meiosis based on the dorsoventrality of the chromosomes established in them by the activity of the kinetochore, as was already outlined in a previous paper (Piza, 1942).

The kinetochore is an universal morphological particularity of the chromosomes as important for them as the nucleus for the cell (Piza, 1941a). Without a kinetochore chromosomes can not exist. Besides any other function which may be ascribed to it, the kinetochore must be considered as the kinetic center of the chromosomes.

Chromosomes attract and repel one another as wholes (cytologically demonstrated by Piza, 1942). Repulsion seems to be a permanent property of chromosomes (Darlington, 1937; Koller, 1934; White, 1937), while attraction is to be regarded as a new property confined to homologous chromosomes periodically conferred on them by the kinetochore. At prophase of meiosis the attraction power developed between homologous chromosomes overcoming the repulsion power, they approach one another. The attraction power does not suppress the opposite one which continues to prevail among non-homologous chromosomes. In the beginning, the activity of the kinetochore seems to confer on the whole chromosome a weak and more or less generalized power of attraction, so that pairing begins by chance at any part of the chromosomes. Later, the attraction power without disappearing

from the rest of the chromosomes becomes concentrated in the kinetochores, thus promoting their coincidence. Due to this primary coincidence all other parts of the chromosomes sooner or later finish by coinciding too. In the course of the development of the chromosomes the kinetochore becomes localized at one of their sides, establishing in this way a clear dorso-ventrality in the chromosomes, cytologically demonstrable (Piza, 1942). Hereafter chromosomes attract one another by the ventral (kinetochore) side. The attraction power is stronger at the kinetochore than at any other part of the ventral side of the chromosomes. The formerly generalized repulsion power, which never disappears from the chromosome body, is now displaced to the dorsal side, remaining stronger at the kinetochore region. In chromosomes like those of Tityus provided with a kinetochore at each end the attraction power seems to be effective along the entire ventral side, in consequence of which the paired elements can untwist till they become perfectly parallel. In metaphase, chromosomes are united venter by venter. The poles attract the ventral side of the chromosomes and repel the dorsal one. At metaphase, the ventral side of the chromosomes being unexposed to the polar attraction, the poles, acting on their dorsal side, repel them to the plane of the equator. In anaphase the disorganization of the chromosomes, sometimes very pronounced at the end of this phase, begins by a decrease in the activity of the kinetochore and consequently of the ventral side, so that, when the mutual attraction power of this face becomes smaller than the attraction power the poles exert on it, the chromosomes rotate. Then, offering the ventral side to the polar attraction and facing one another with the repelling dorsal sides, the chromosomes separate and anaphase proceeds.

In chromosomes provided as ordinarily with a single kinetochore the kinetic activity of the ventral side may be more or less concentrated in it, so that separation begins at this point.

Secondary spindle fibers, that is, fibers without direct connection with the kinetochore, may be formed between the poles and a more or less extensive area of the ventral side of the chromosome, in accordance with the activity conferred to this side by the kinetochore. In Tityus, Steatococcus and in the X-chromosome of Protenor the whole ventral side shows kinetic activity and determines spindle fiber formation. Most chromosomes with a single kinetochore are attached at the extremity of a bundle of fibers, parallel or spread out like the sticks of a very delicate fan, revealing in this way a certain kinetic activity in the neighborhood of the kinetochore.

The hypothesis that each chromosome is dorsoventrally differentiated is not new. The first investigator to propose such an interpretation was Cooper (1938), who suggested that "the synapsing chromosomes are bilateral in organization, *i.e.*, constructed in such a manner that each chromosome possesses but one, limited, pairing surface."

Schrader (1940) approached very closely to the present interpretation of mitosis when he wrote: "So far as I can see, there are just two ways of escape from the difficulty: one, that the negative charge of either chromosome or center changes to a positive one or else becomes weak or neutral, while the movement is going on; the other, that, unlike the main mass of the chromosome, the kinetochore is positive and thus is not repelled by the center."

Pease's experiments, demonstrating that the rate of chromosome movement in anaphase decreases with the fluidification of the spindle, do not contradict the idea of an attraction exerted by the poles, considering that no modifications introduced by hydrostatic pressure, either into the poles or into the chromosomes and capable of altering the movement of the latter, were taken into account. Likewise, Shimamura's investigation on the effects of centrifugal force on nuclear division (1940) do not contradict the conclusions of the present paper with regard to the uselessness of the spindle fibers in attaching

the chromosomes to the poles or in moving the chromosomes, since what the experiments have really brought into evidence was merely the great power of the force which maintains the kinetochore turned toward the poles even when the body of the chromosome has been projected to the centrifugal side. The effect of centrifugation on material previously submitted to chloralhydrate and colchicine treatment has demonstrated in its turn that in the treated cells, if the poles did not suffer any modification in their attraction power (?), the chromosomes at least have lost the faculty of reacting to the influence of the poles, becoming incapable of moving and consequently of producing spindle fibers.

The Behavior of the Chromosome Fragments of Steatococcus

This question will become more comprehensible when we get a more extensive information about the structure and function of the kinetochore. For the moment we can only consider the kinetochore not yet marked by the presence of a granule as representing a more primitive state in the story of this element. In this case we can assume that the kinetochore region of the chromosome may be represented in the beginning of prophase by a more or less extended area which would be more and more restricted as the nucleus proceeds in its mitotic differentiation. Every chromosome fragment of Steatococcus which behaves like an unbroken chromosome must have at least a kinetochore originated from fragmentation of the kinetochores of the normal chromosomes, which must have occurred at a time when the chromosomes and most probably the kinetochores still were in a state of great distention. This assumption is in full agreement with Schrader's (1939) suggestion "that there is some reorganization of the kinetochore at every mitotic cycle and that functional fragments, such as reported by McClintock, originate at a time when the final form shown in metaphase has not yet been assumed."

Chromosome fragments associated with a whole kineto-chore or with a large piece, would behave like normal chromosomes, their parallelism at anaphase being accountable for by an uniform distribution of the kinetic activity along their entire ventral face. The chromosome fragments provided with a rather small piece of the kinetochore, due to the insufficiency of it, would lag and finally would be lost. Finally, fragments of chromosomes entirely unprovided with kinetochore, might show some kinetic activities due merely to the normal repulsion power of the chromosomes still present in them, but sooner or later would vesiculate and disappear.

Summary

The meiotic chromosomes of *Tityus bahiensis*, provided with a kinetochore at each extremity, are attached at the spindle long before metaphase is reached. Being still twisted and bent at that time, they have to perform many varied movements in order to assume their rod-shaped metaphase form. Since the spindle fibers are not altered in any way by the movements of the chromosomes it was concluded that they are nothing else than the inevitable consequence of a very feeble coagulation process developed continuously between the kinetochores and the pole, following exactly the line connecting these points and without any influence in moving the chromosomes.

Steatococcus chromosomes and the X-chromosome of Protenor, like Tityus chromosomes, are considered as being provided with two terminal kinetochores. An explanation based on the dorso-ventrality of the chromosomes is proposed for the presence of fibers along the entire polar face of the chromosomes.

The presence of a kinetochore at each end makes intelligible the fact that the X-chromosome of Protenor, in the second meiotic division of the spermatocytes, moves parallel to the spindle axis toward one of the poles.

A new theory of meiosis, based on the dorso-ventrality of the chromosomes, is presented.

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The Genetics of the Mouse. By HANS GRÜNEBERG. Cambridge: At the University Press; New York: The Macmillan Company, 1943: i–xii, 1–412, pls. 1–14, figs. 1–43. $7.00.

THIS comprehensive and authoritative survey of the genetics of the house-mouse (*Mus musculus* and *M. bactrianus*) will be of great value to all those numerous investigators who utilize this common animal in their laboratories. It will be of particular interest to geneticists, anatomists, physiologists and pathologists. More is known about the heredity of the house-mouse than about that of any other laboratory mammal. The number of inherited variations that have been discovered in this rodent is very considerable, but because of the uncertain status of many characters the exact number of known genes cannot be stated.

Practically every part of the body of the house-mouse exhibits hereditary modifications of one sort or another. The total body size may be decreased or increased; the tail, limbs, or external ears may be reduced in size or variously deformed; the eyes may be rudimentary or absent; and there may be cleft lip and palate. There are many striking modifications of the pelage leading to the production of numerous variations of color, including various types of spotting with white; the hair may be variously curled or misshapen, or may be absent during a part of the life-history, leaving the animal naked. The bones may be greatly modified, especially in the head, limbs, and tail; in one strain there is congenital absence

of the tibia. Inherited abnormalities have been described for the pituitary, thyroid and adrenal glands, for the urinogenital system, for the blood and blood-forming organs, and for other internal organs. There are inherited differences in resistance to certain types of disease, including cancer, and also differences in serological reactions. The brain and organs of special sense also exhibit a number of inherited abnormalities and certain types of blindness and deafness are thereby produced. There are inherited differences in behavior, in learning ability, in wildness and savageness, and in social behavior, but the precise genes involved in most of these psychological differences have not yet been analysed.

A brief but valuable summary on "The Genetics of Cancer in Mice" is contributed in an appendix by C. C. LITTLE and P. A. GORER.

"These structural variations and pathological processes show a considerable likeness to human affections, and the day will come when human pathologists will realize the value of the inherited diseases of the mouse in solving problems of human pathology, where observation is often difficult and experimentation usually impossible. Hitherto, the majority of human pathologists are hardly aware of this promising material . . ." Investigations in anatomy, embryology, physiology, pathology and psychology would often be benefited by the use of an animal such as the house-mouse, whose genetics have been at least in part worked out. The similarities of the house-mouse to man in many of its characters make it particularly appropriate for investigations in these fields. The genetics of other kinds of laboratory mammals have been worked out less thoroughly than that of the house-mouse. The author fails to point out, however, that some of the other laboratory animals would, because of their larger size, be more suitable than the mouse for certain types of studies.

It is surprising that inherited epilepsy has not yet been described for the house-mouse, although inherited types

of other behavior defects, such as waltzing, shaking, and circling have long been known. A number of inherited types of abnormal behavior, sometimes including convulsive seizures, are known for *Peromyscus* and for the house-rat and it is the belief of the reviewer that careful investigation will discover similar types of hereditary convulsive behavior in the house-mouse.

Among some of the more interesting genes are those that affect several different parts of the body. For instance, the gene for siderocyte anemia often produces also a flexed tail and a white spot on the belly. A number of the other genes are known to produce at least two entirely different sorts of effects. Although geneticists are aware that every gene probably affects several different organs or indirectly the whole organism, it is not always easy to demonstrate that such widely divergent effects actually are produced by the same gene.

A group of genes of particular interest to geneticists are those that change in dominance depending on their genetic background. The piebald gene W, for instance, may be either dominant, semidominant or recessive, depending upon the modifiers with which it is associated.

Phenotypic effects that appear to be similar may be and frequently are produced through the action of entirely different genes. Warning is therefore given against attempts to homologize genetic characters in different animals on the basis of the phenotypic effects alone. In the opinion of the reviewer, this warning is particularly pertinent in the field of human genetics. It is never safe to assume that a similarly appearing character that occurs in two independent human kindreds actually is due in both cases to the same gene. Much controversy about the mode of inheritance of human traits can be avoided if this warning is kept in mind.

The number of chromosomes in the house-mouse is forty, of which one pair form an XY combination related to sex. There are twenty possible linkage groups. One of the genes concerned in the transplantability of a can-

cerous tumor is known to be sex-linked, but no gene producing a visible effect has been demonstrated to be located on the X-chromosome. By linkage tests eight of the autosomes have been demonstrated each to carry at least two genes. Only one mouse chromosome, however, has been definitely proved to be the carrier of as many as three genes.

A comprehensive bibliography of 1,141 titles is given. This is arranged according to subject, but the contributions of individual authors may be found through the author index.

The publication of such a book as this in war-time England is a notable achievement. Nevertheless, the price of $7.00 for a book of this size is greatly in excess of that usually asked for books of the same character printed in the United States. The high price is to be regretted, for this will prevent the purchase of the work by many of the persons who would profit by its possession.

<div align="right">Lee R. Dice</div>

Joseph Grinnell's Philosophy of Nature Selected Writings of a Western Naturalist. Berkeley and Los Angeles: University of of California Press, 1943: i–xv, 1–237, 13 figs. and pls. $2.00.

A splendid idea, a worthy tribute to a great naturalist, a significant contribution to the philosophy of evolution!

To the end of his days Joseph Grinnell kept too busy with special researches to yield to requests that he write books which would bring together and make generally available his highly respected views on the relations between organisms and their environment. He had, however, always been a penetrating and clear thinker as well as an exceptionally productive researcher, and he had scattered through his special reports wise generalizations which had developed from these researches and thoughts. Alden H. Miller and other devoted students and colleagues found it possible to supply the long-felt need, by selecting from Grinnell's large bibliography a series of papers, and sections of others, that reflect the views of this master

naturalist on general systematics, ecology, zoogeography, conservation and speciation.

"Joseph Grinnell's Philosophy of Nature" takes the form of a series of essays, delightfully written and chronologically arranged; interesting enough to induce the reader to complete the book, yet sufficiently short and independent to permit the separate reading of the items, during the fragments of time that these busy days leave us for general reading. Though the extracts are of works published during one third of a century (1903 to 1936) the views expressed are by no means outmoded. Grinnell consistently thought ahead of his time. It is a source of surprise to note the date of original publication of viewpoints that would reflect the keenest modern thought on evolutionary problems.

Grinnell approached the problem of evolution through the analysis of the environmental relations and the characters of subspecies—species caught in the act of evolution. He held that each form evolves by natural selection, becoming thoroughly adapted to a particular ecological niche, and that its differentiation is rigidly conditioned by isolation. The following points are particularly stressed:

> The course of organic evolution has been molded and is being molded by environmental circumstance. In one sense this is directed evolution—orthogenesis of a kind. . . . Plant-animal communities . . . have been subject to evolutionary processes quite as definitely as discrete species.

Chimpanzees A Laboratory Colony. By ROBERT T. YERKES. New Haven: Yale University Press, 1943: i–xv, 1–321, frontisp. + pls. 1–63, figs. 1–24. $5.00.

THE story of the chimpanzee is entrancingly told, but so comprehensively that the recitation of experiments, observations and anecdotes has been cut to tantalizing briefness. After finishing Yerkes' latest book one will be sorely tempted to study other books and articles on the great apes, and if blocked in this desire by the high premium which these overcrowded days place on general reading, he may hold toward the author feelings other

than those of unmitigated gratitude. If so, the times are to be blamed.

Yerkes treats chimpanzees as animals preadapted to the solution of problems by preceptual, presumably ideational, responses, at time "by a clear vision of the solution." In their minds as well as in their bodies and their diseases he looks on these apes as almost human. In their ways of life and in their mental reactions he sees, at least as a glimmer, the basis of the human essentials—socialization, symbolism, language and culture. Yet he has obviously striven to understand the chimpanzee mind as a distinctive entity. He stresses in particular the importance of the positional arrangement or relative direction of objects in the memory and delayed responses of this ape. From his psychobiological viewpoint Yerkes presumably joins most modern zoologists and anthropologists in regarding the chimpanzee as our cousin rather than as our ancestor.

The book is loaded with interest for the zoologist as well as the psychologist, the pathologist, the animal keeper and the sociologist. It contains a wealth of sound bilological philosophy. Thus, in treating the social development of the individual, he is convinced "that heredity and environment are inseparables, whose importance varies for different patterns of response and phases of organic development."

Outstanding among many treats is the autobiographical account of the early conception, the long promotion, the final consummation and the full justification of the Yale Laboratories of Primate Biology, recently placed on a continuing basis and very appropriately renamed the Yerkes Laboratories. Yerkes promises science, in the final statement, that his release from the administration of this already amazingly productive institute "is not the end, for essentially preparatory work now can give place largely to fundamental research." May he be given many years to further his own analyses of primate culture, and to aid him in his expressed desire of contributing to the foundation of human engineering!

Speaking of Man A Biologist Looks at Man. By MICHAEL F. GUYER. New York and London: Harper and Brothers, 1942: 1–321. $3.50.

CONGRATULATIONS are due to Professor Guyer, for his help in dispelling the idea that noted research biologists are encloistered recluses who have lost their ability to associate and converse with the common man. He speaks well and understandingly. Some passages, indeed, are superb combinations of deep, clear thinking with satire and wit. Had such style been sustained, the author's reputation would no doubt have passed beyond that of a master of popular scientific style to that of a literary genius.

What Dr. Guyer speaks about so well is not merely, in fact in rather small part, the biology of man. His ten talks cover a broad range of subjects: Biology and the Happy Life, Science and Its Critics, Man's Place in Nature, The Rise of Intelligent Behavior, Managing Our Minds, The Endocrine Control of the Body, Sex, Democracy as a Biological Problem, The Educated Failure, Man's Search for the Ideal. These essays constitute a frank philosophy of human life, that should lead many to a trurer and more wholesome outlook.

NOTICES OF NEW BOOKS

General Zoology. By TRACY I. STORER. New York and London: McGraw Hill Book Co., 1943: i–xii, 1–798, 5 col. pls., 551 figs. $3.75. This is a marvelously informative text and general reference book on zoology. Perhaps its most impressive features are the many new illustrations, which strikingly clarify the text discussions and must surely incite the interest of any worthwhile student. The eye is particularly arrested by the colored plates which boldly exhibit the internal anatomy as semi-transparencies and by the ecological diagrams which illustrate differential habitat selection. The whole treatment, first under topical and then under systematic arrangement, is remarkably thorough and well balanced. Many zoologists of the future will be better zoologists for having received their inspiration and early training from Tracy Storer's book.

Animal Breeding Plans. Second Edition. By Jay L. Lush. Ames: Iowa State College Press, 1943: i–viii, 1–437, figs. 1–50. $3.50. Lush's comprehensive and authoritative advanced textbook on animal breeding passes into its second edition. Changes are greatest in the chapters on Genetic Basis of Variation and on Family Structure of Populations. The theoretical as well as the applied aspects of animal breeding are dealt with, and particular stress is laid on the problems involved in the improvement of livestock. The book is of great interest and value, however, to the general zoologist, the geneticist and the student of speciation. This is particularly true of the analyses of the nature of differences between breeds, the operation of selection, the structure of populations, inbreeding, line breeding and outbreeding—in all of which the statistical genius of Sewall Wright has been heavily relied upon.

American Society of Naturalists

As a result of its annual balloting the American Society of Naturalists has elected the following officers to serve for the year 1943: H. J. Muller, Amherst College, *President;* B. M. Duggar, University of Wisconsin, *Vice-President;* A. C. Kinsey, Indiana University, *Secretary;* M. R. Irwin, University of Wisconsin, *Treasurer.*

The following persons were elected, on account of outstanding achievement in biological research, as members of the society: Ernest C. Abbe, LeRoy Abrams, F. A. Beach, J. P. Bennett, James Bonner, Ralph Buchsbaum, Earl O. Butcher, Fred K. Butters, Wanda K. Farr, David R. Goddard, Karl C. Hamner, Edwin R. Helwig, Hope Hibbard, Theodore L. Jahn, John S. Karling, Stewart A. Koser, Alfred M. Lucas, Gordon Marsh, H. M. Parshley, Frederick V. Rand, P. L. Risley, Ralph Singleton, J. M. Webber, S. H. Yarnell.

SHORTER ARTICLES AND DISCUSSION

INHERITANCE OF MOTTLED EARLOBES AND STUBS IN RHODE ISLAND REDS[1]

In recent years the appearance of mottled earlobes in Rhode Island Reds bred for high fecundity has been rather widespread. The amount of white mottling varies widely between individuals from a few whitish areas to large areas in which the earlobe is essentially white.

Warren (1928) observed that many of the so-called red earlobe breeds do not breed true for red earlobes. In his study of crosses between Rhode Island Reds and Leghorns there was evidence of several factors operating to modify earlobe color, probably two autosomal genes, but there was no evidence of sex-linked genes affecting the amount of white in the earlobes of Rhode Island Reds.

The appearance of mottling in the earlobes of Rhode Island Reds is objectionable according to breed standards, and breeders have had considerable difficulty in eliminating it from their flocks. Just why this variation should become more prevalent during the last decade is not clear. The possibility exists, however, that in breeding for high fecundity there have been more related matings which would lead to genetic segregation.

Stubs are rather common in many breeds that normally lack any evidence of feathers on shanks or toes. The appearance of down between the toes or on the shanks of Rhode Island Red chicks at hatching is not uncommon, but this down does not usually persist longer than a few days. There is no evidence in our flock that down on the shanks or toes is a precursor of stubs at maturity. The appearance of down on the toes and shanks that was observed by Warren (1930) in White Leghorn adults was not observed in our Rhode Island Reds. Stubs in our stock consisted of short feather quills on the shanks or between the toes.

Lambert and Knox (1929) present a review of the literature on shank feathering which need not be repeated here. They are inclined to agree with Serebrovsky (1926) that probably four genes are concerned in shank feathering and that there is evidence that recessive genes may be responsible for stubs in clean shanked breeds.

[1] Contribution No. 464 from the Massachusetts Agricultural Experiment Station.

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EXPERIMENTAL DATA

This study was undertaken to discover the mode of inheritance of mottled earlobes and stubs within the Rhode Island Red breed. The investigation began with the generation hatched in 1935 and carried through eight generations concluding in 1942. During this period 7 different sires were mated to 58 dams to produce 820 offspring that reached the age of six months. Records were made at hatching for down on the shanks or toes and at the approximate age of six months for stubs and earlobe color in the complete families.

Each generation was sired by a single male mated to several hens. The second and third generations were sired by two brothers from the first generation so that there was some inbreeding. An outside male with red earlobes and stubs was used to produce the fourth and fifth generations. The last three generations were each produced by a son of the preceding generation so that inbreeding was practiced. The sires used in 1940 and 1941 had mottled earlobes and stubs and the sire used in 1942 had red earlobes and clear shanks.

INHERITANCE OF MOTTLED EARLOBES

In this experiment some inbreeding was practiced in that sons were often mated back to their dam and were also mated to their half sisters and one sire that was used for two years was mated to several of his daughters. A careful study was made of the phenotypes produced from mating normal sires to normal dams, normal sires to mottled dams, mottled sires to normal dams and mottled sires to mottled dams.

There was good evidence that inbreeding increased the incidence of mottling, but there was evidence that the mottled condition may not always appear at the age of six months and may be observed in the same individuals in the spring of the second year when the birds are about twelve months old.

Common phenotypic ratios observed were: 9 to 7 and 3 to 1 when normal phenotypes were mated. When one parent was normal and the other mottled the common ratios were: 1 to 1, 3 to 5 and 1 to 3. When both parents showed the mottled condition the usual phenotypic ratio of the offspring was 1 to 1. The fact that matings where one parent was normal and the other mottled often gave 1 normal to 3 mottled indicates that more than one recessive gene is concerned. When mottled is mated

to mottled the phenotypic ratio of the offspring is often 1 to 1. This is evidence that either of two recessive genes may produce mottling. A single recessive gene would not give phenotypical ratios like those observed and there was no evidence of sex-limited or sex-linked inheritance.

In Table 1 the data are summarized by phenotypes for the different matings to save space. The most probable genotypes of the parents are indicated.

TABLE 1

COMBINED RESULTS OF MATING FOR MOTTLED AND NORMAL EARLOBE
COLOR, 1935–1942

5 Normal Sires ($R_1r_1R_2r_2$) × 19 Normal Dams ($R_1r_1R_2r_2$)

Sons		Daughters			Totals	
Normal	Mottled	Normal	Mottled		Normal	Mottled
136	67	100	94		236	161
				Expect	223	174

5 Normal Sires ($R_1r_1R_2r_2$) × 22 Mottled Dams ($R_1r_1r_2r_2$)

Sons		Daughters			Totals	
Normal	Mottled	Normal	Mottled		Normal	Mottled
67	89	45	107		112	196
				Expect	115	193

2 Mottled Sires ($R_1r_1r_2r_2$) × 8 Normal Dams ($R_1r_1R_2r_2$)

Sons		Daughters			Totals	
Normal	Mottled	Normal	Mottled		Normal	Mottled
17	20	34	18		51	38
				Expect	33	56

1 Mottled Sire ($R_1R_1r_2r_2$) × 4 Mottled Dams ($r_1r_1R_2r_2$)

Sons		Daughters			Totals	
Normal	Mottled	Normal	Mottled		Normal	Mottled
8	6	7	5		15	11
				Expect	13	13

From the matings of birds with normal red earlobes there were produced 236 normal to 161 mottled offspring or a 9 to 7 ratio. This would indicate that two recessive genes r_1 and r_2 must be present in the stock. Each of these recessive genes must be covered up by its allele to give the normal red condition.

When normal males were mated to mottled females the offspring showed 112 normal to 196 mottled. This ratio would be approached if the sires were heterozygous for both genes and the mottled dams were pure for one recessive gene and heterozygous for the other.

The reciprocal cross mottled males on normal females gave too few mottled female offspring but a reasonable approach to expectation.

A single mottled male mated to four mottled females gave 15 normals to 11 mottled while equality was expected. This mating gives further evidence that either of two recessive genes may produce mottling. The fact that normal × normal gives both

normal and mottled indicates the dominant nature of genes for red earlobe. There is no evidence in our data of sex-linked genes being concerned in the mottled earlobe condition.

INHERITANCE OF STUBS

The stock of Rhode Island Reds used in the study of mottled earlobes was also classified with respect to the presence or absence of stubs in complete families at six months of age. This study began in 1936 and includes·seven generations.

Stubs appear to behave in inheritance in identical fashion with the mottled earlobe condition, although the two characters are entirely independent. A careful study was made of phenotypic ratios from different types of matings through a seven year period. It was noted that stubs can be recognized by careful examination at six months of age. They show a tendency to disappear in some birds during the first laying year. In this study all records were made at six months in both males and females.

In Table 2 all data are grouped according to phenotype and parents.

TABLE 2

COMBINED RESULTS IN MATINGS FOR CLEAR SHANKS AND STUBS—1936–1942

3 Clear-Shanked Sires × 24 Clear-Shanked Dams

	Sons		Daughters			Totals	
Clear	Stubs		Clear	Stubs		Clear	Stubs
162	20		150	16		312	36

1 Clear-Shanked Sire ($S_3S_3S_4S_4$) × 3 Dams with Stubs ($s_3s_3S_4S_4$)

	Sons		Daughters			Totals	
Clear	Stubs		Clear	Stubs		Clear	Stubs
2	2		4	1		6	3
					Expected	7 to	2

3 Sires with Stubs ($s_3s_3S_4s_4$) × 18 Clear-Shanked Dams ($S_3S_3S_4S_4$)

	Sons		Daughters			Totals	
Clear	Stubs		Clear	Stubs		Clear	Stubs
86	40		110	34		196	74
					Expected	203 to	67

2 Sires with Stubs ($S_3S_3s_4s_4$) × 4 Dams with Stubs ($s_3s_3S_4s_4$)

	Sons		Daughters			Totals	
Clear	Stubs		Clear	Stubs		Clear	Stubs
12	12		9	8		21	20
					Expect	20.5 to	20.5

Stubs in Rhode Island Reds appear from the above data to be produced by either of two recessive genes s_3 and s_4 that are not sex-linked. For example, the mating of 1 clear-shanked sire to 3 dams with stubs gave 6 with clear shanks to 3 with stubs which closely agrees with expectation. Three sires with stubs when mated with 18 clear-shanked dams gave 196 clear to 74 with

stubs while the expectation was 203 to 67. Mating two sires with stubs to 4 females with stubs gave 21 clear to 20 with stubs when equality was expected. A deficiency of females showing stubs occurs in our data, as was observed by Lambert and Knox (1929). It is our belief that this character is partially sex-limited.

DISCUSSION

Within the Rhode Island Red breed evidence indicates that the mottled earlobe condition is produced by two recessive autosomal genes designated as r_1 and r_2. No evidence is available on possible cumulative effects of these genes but the extent of mottling varies rather widely. Because of the nature of inheritance it is very difficult to entirely eliminate the mottled earlobe condition from Rhode Island Reds.

Apparently stubs are inherited in a similar fashion to the mottled earlobe condition. Two independent recessive autosomal genes are indicated, genes s_3 and s_4. Cumulative effects on the extent of leg feathering produced by these genes have not been determined. This undesirable character is also very difficult to eliminate from the flock.

. For purposes of completely eliminating either mottled earlobes or stubs in Rhode Island Reds, an inbreeding program offers possibilities of uncovering these recessive genes. There was no evidence of any linkage between any of the four genes.

CONCLUSIONS

Mottled earlobes in Rhode Island Reds appear to be produced by two recessive autosomal genes.

Stubs in Rhode Island Reds seem to depend upon two recessive autosomal genes.

No evidence of linkage was observed between genes for mottled earlobes and genes for stubs.

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RELATIONSHIP BETWEEN THE LENGTH AND THE WEIGHT IN THE SNAPPING TURTLE *CHELYDRA SERPENTINA* LINNAEUS[1]

IT is now well established for fishes that weight (W) usually may be expressed as a function of length (L) by means of the equation, $W = C L^n$, where C and n are empirically determined constants. To our knowledge, however, such a relationship has not heretofore been shown for any chelonion species. Data are here presented on the length and weight of snapping turtles (*Chelydra serpentina*) and are analyzed to show the nature of the relationship between length and weight for this species and to provide a practical means for approximating weight when length is known and *vice versa*.

The measurements that we have used were made on 151 common snappers from the Lower Peninsula of Michigan. These specimens were collected in lakes, ponds and streams during the summers of 1937 and 1938, mostly by means of traps. All were weighed alive, at the time of capture, on a spring balance accurate to the nearest ¼-pound. The length, also obtained at time of capture, is the greatest over-all length of the carapace as measured horizontally through it by means of a large caliper, accurate to the nearest ⅛-inch.

Preliminary analyses failed to reveal sexual dimorphism for the features studied and for our purposes sex is disregarded. Males numbered 74, females, 77.

The equation, $W = C L^n$, reduced to logarithmic form, may be written: $\log W = \log C + n \log L$. The equation may be applied to observational data, therefore, by fitting a straight line to the logarithms of the empirical lengths and weights. In the present study the equation was not fitted to the data for the individual turtles but rather to the average lengths (in millimeters) and weights (in grams) of turtles within half-inch intervals of length.

The value of C may be computed from the formula:

$$\text{Log } C = \frac{[\Sigma \log W \cdot \Sigma (\log L)^2] - [\Sigma \log L \cdot \Sigma (\log L \cdot \log W)]}{[N \cdot \Sigma (\log L)^2] - (\Sigma \log L)^2} \quad (1)$$

Where N = the number of points (15) in the empirical data. The value for the logarithm of the constant was found to be – 3.789234.

The value of n was found to be 3.06383, as determined by the application of the formula,

$$n = \frac{\Sigma \log W - N \cdot \log C}{\Sigma \log L} \qquad (2)$$

The length–weight equation for snapping turtles from the Lower Peninsula of Michigan is therefore:

$$\log W = -3.789234 + 3.064 \log L \qquad (3)$$

or

$$W = 1.625 \times 10^{-4} \, L^{3.064} \qquad (4)$$

Since the value of n is very near to 3, the length–weight relationship of this turtle would appear to approximate the "cube law" fairly closely. With a few exceptions, the n in equations fitted to the length–weight data of fishes has not deviated far from 3, and values above that figure have been more numerous than exponents less than 3.

Comparisons between empirical weights and weights calculated by means of equation (3) may be had from the data of Table 1

TABLE 1

LENGTHS AND WEIGHTS (AVERAGES FOR HALF-INCH SIZE GROUPS) OF SNAPPING TURTLES AT CAPTURE AND THEORETICAL WEIGHTS AT THE VARIOUS LENGTHS AS COMPUTED FROM THE LENGTH–WEIGHT EQUATION

Number of Turtles	Length (Inches)	Weight (pounds)		Length (Milli-meters)	Weight (grams)	
		Actual	Calculated		Actual	Calculated
2	7.75	4.3	3.8	197	1,873	1,740
4	8.25	5.3	4.7	210	2,404	2,116
16	8.75	5.8	5.5	222	2,631	2,509
20	9.25	6.7	6.6	235	3,039	2,987
25	9.75	7.4	7.8	248	3,357	3,523
25	10.25	8.7	9.0	260	3,946	4,071
16	10.75	9.6	10.4	273	4,355	4,728
9	11.25	11.3	11.9	286	5,126	5,420
12	11.75	13.3	13.6	298	6,033	6,184
1	12.25	15.5	15.4	311	7,031	6,999
8	12.75	16.9	17.6	324	7,666	7,990
10	13.25	18.7	19.9	337	8,482	9,014
2	13.75	22.0	22.1	349	9,979	10,034
1	14.25	25.0	24.7	362	11,340	11,220
1	14.75	30.0	27.6	375	13,608	12,505

and Fig. 1. In general, the theoretical length–weight curve fitted the empirical data rather well. Fairly large discrepancies occurred at some intervals, it is true, but these irregularities can be explained satisfactorily as the result of the small number of turtles in the collection. Six of the 15 half-inch intervals were represented by less than five turtles each, and three of these six were represented by only one specimen.

It is concluded, therefore, that the length–weight relationship of snappers from the Lower Peninsula of Michigan over the

length range of 7.5 to 15 inches may be expressed satisfactorily
by the general equations (3) and (4). It is possible also that the
extrapolation of the curve for a reasonable distance outside this
length range may give satisfactorily accurate results. The equa-
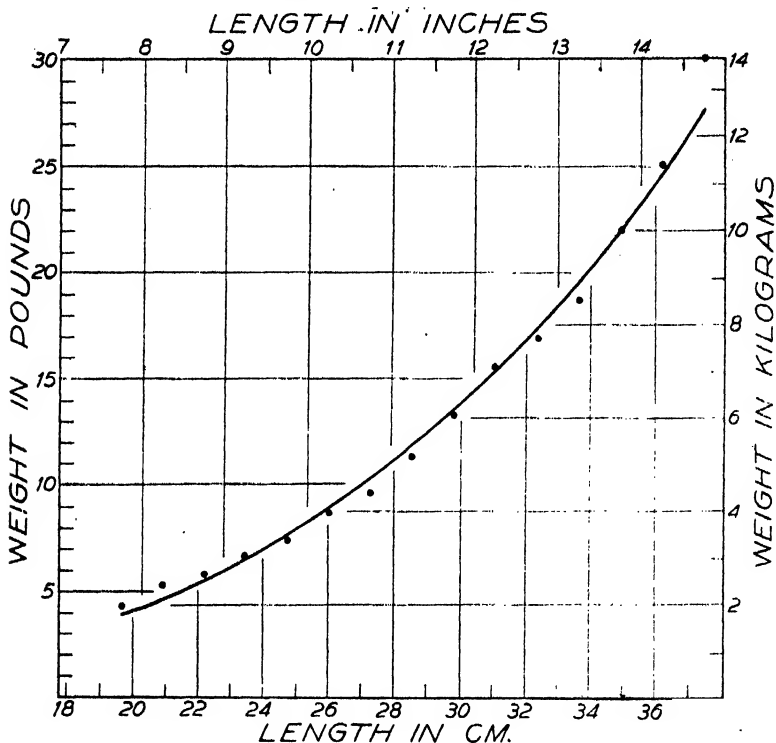
FIG. 1. Length–weight relationship of the snapping turtle in the Lower
Peninsula of Michigan (sexes combined). The curve is the graph of the
equation fitted to the length–weight data and the dots represent the empirical
averages of length and weight.

tion and graph therefore provide useful methods of estimating
weight when length alone is known.

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PARTIAL OOSORPTION AS A POSSIBLE CAUSE OF DIPLOID MALES IN MICROBRACON HEBETOR

THE occurrence of sex reversal in newly hatched larvae of *Tribolium* by means of starvation (Holdaway, 1930) and in the larvae of certain Hymenoptera by means of parasitization (Wigglesworth, 1940) indicates that the extraction of nutriment from ripe ovarian eggs subjected to the process of oosorption may have a similar effect if such eggs are fertilized and deposited before their viability is destroyed.

In the Hymenoptera, biparental males are known to occur only in *Microbracon hebetor* (Whiting, 1935). Such males are not sex-reversed females, according to inheritance studies made by Whiting (1935), if the occurrence of biparental males is the result of some condition correlated with the interbreeding or close relations of the parasites. However, it was observed by Whiting that in *M. hebetor* there is a definite correlation between the occurrence of non-hatching eggs and biparental males; the biparental males being produced by close-bred females which produce a much higher percentage of non-hatching eggs than either the unmated females or females mated with unrelated males. The increase in percentage of non-hatching eggs from close-bred females and the decrease in percentage of biparental offspring indicates that in such females there is a diminution in responsiveness to oviposition stimuli and a corresponding increase in the absorption of the ripe ovarian eggs.

In ectoparasitic species, oosorption probably proceeds with greater rapidity than oogenesis (Flanders, 1942). Slowness in response to oviposition stimuli may result in the deposition of slightly absorbed eggs of low viability. A decrease in oviposition rate may account for the fact noted by Whiting (1940) that the percentage of nonhatching eggs deposited by *M. hebetor* increases with the age of the ovipositing female.

Whiting (1940) points out that in *M. hebetor* embryonic development occurs in almost every nonhatching egg. Consequently, it seems probable that eggs that have not regressed beyond a certain point may hatch and, if able to feed on their host, complete their development.

There exists, therefore, the possibility that individuals from slightly regressed fertilized eggs may have undergone sex reversal prior to feeding on the host as a result of undernourishment during embryonic development.

In the ectoparasite *Melittobia chalybii*, in which close breeding is normal, a high percentage of nonhatching eggs is often observed. In unmated females, however, the percentage is much greater since, unlike *Microbracon hebetor*, mating is a prerequisite of normal oviposition. Females mated with males of a different species also oviposit normally. Since unmated females, females mated to males of another species and normally mated females may produce equal numbers of male offspring, it seems evident that in *M. chalybii* biparental males rarely, if ever, occur (Schmieder, 1938). In this species the relation between oviposition and oosorption may be such that regressed eggs capable of development are not deposited.

It is possible that the production of diploid males in the Hymenoptera is a specific phenomenon, correlated with rate of oosorption in the ovary.

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A CHEMICAL AND HISTOLOGICAL STUDY
OF THE FEATHER PIGMENTS OF
THE DOMESTIC FOWL[1]

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MANY studies have been conducted in an effort to determine the intermediate reactions initiated by the discrete genes which culminate in the phenotypic expression. Much of such investigation has been concerned with the development of pigments in various organisms. The choice of color for studies of this type is probably due to the great number of variations and particularly to the striking differences observed. Some workers also consider the path between the initial gene action and the observed color to be relatively direct, which may or may not be true.

Pigment substances are very stable compounds which do not lend themselves readily to chemical analysis. Due to this fact, knowledge of the chemical and physical nature of the pigments is very limited. Progress in the attack on the general problem of pigmentation would be expedited by any additions to our knowledge of the nature of the end products of gene action. With this fact in mind, the following investigation was initiated with the intent of further clarifying the nature of the end products involved in producing the color patterns of the fowl. Using the feathers from a large number of breeds and

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varieties of poultry, a survey was made in which histological, spectrophotometric, and solubility observations were recorded and analyzed.

HISTOLOGICAL STUDIES OF FEATHER PIGMENTS
Material and Methods

Feathers from 37 different breeds and varieties of poultry, including red, black, blue, buff, white and various gold and silver color patterns, were examined. In order to have more uniform samples, cushion feathers were utilized in most cases. In a few instances, however, feathers from other regions were studied if they showed coloration which seemed to merit consideration. The feather samples were supplied by specialized breeders in this country and Canada.

Whole mounts of web and fluff barbules were prepared, in order to study the distribution of the pigment and its granular nature in the natural condition. The desired portion of the feather was placed in xylol for clearing. This process required several weeks for the barbs but since no medullary air spaces were present in the barbules, they were completely cleared in only a few days. Several more days were required for infiltration with balsam. In order to obtain a satisfactory lateral view of the barbules they were removed from the barbs. This was accomplished by placing balsam infiltrated barbs on a slide under a binocular microscope and carefully severing them at their bases by using a razor blade. More balsam was then added and a cover glass applied.

Cross sections of feathers in general proved much less satisfactory for a study of feather pigments than the whole mounts. Many such sections were prepared and some observations were made from them. The samples for sectioning were thoroughly cleared in toluene, the time required ranging up to six weeks to penetrate the comparatively thick barbs. This was followed by infiltration with paraffin (m.p. 56–58° C.) in an oven held at 60° C. They were then imbedded in hard paraffin (m.p.

60-62° C.). The paraffin blocks were sectioned at three, five and ten microns after which the sections were mounted in balsam.

Liberated pigment granules were excellent material for determining the size, shape and uniformity of these bodies. Liberation was accomplished by boiling the feathers for two hours in 6N HCl. Longer treatment as suggested by Einsele (1937) did not appear to make the outlines more distinct and was therefore abandoned in favor of the shorter time. In black feathers the keratin was destroyed and the granules became free in the solution. No effect on the granules was noted and the filtrate showed no traces of dissolved pigment. In red feathers, a part of the pigment was dissolved by the acid. A granular pigment remained, however, which was not further affected, even on boiling for as long as two days. All the pigment of buff feathers dissolved quickly in the acid, so that the method could not be applied to this color. The washed granules from black and red feathers were smeared thinly on glass slides and mounted in balsam.

White Varieties

Feathers from white varieties of Orpington, Langshan, Wyandotte, Silkie, Minorca, Plymouth Rock, and Leghorn breeds were examined (Table 1). Definite black pigment granules were observed in all cases. The largest granules were observed in the fluff, being irregularly rod- to spherical-shaped and about 1.1 μ in diameter and from 0.5 μ to 2.0 μ in length (Fig. 14). These granules occurred rather infrequently, either singly or in groups of two to six or occasionally more, and were located in the center of the barbule just proximal to the nodes. The White Plymouth Rock feathers examined showed more of this type of granule than the other breeds studied. Of more frequent occurrence were very minute spherical granules, along with a very few rod-shaped ones, ranging from 0.5 μ in diameter to the limit of visibility (Fig. 9). These granules were largely confined to the base of the barbules

and the sides of the barb to which the barbules were attached. Occasionally single granules or groups of granules of this type were found in the nodes of the fluff. Such granules were found in both web and fluff barbules but were irregular in their occurrence. In some cases they were common in the fluff but rare in the web or vice versa, and frequently only one barbule out of a number showed such granules. The facts that the residue of acid hydrolysis of white feathers contain many of these granules and that they are plainly visible at the base of the barbules in cross sections of white feathers are added evidence of their existence. In a great number of cases where a few definite granules were found in the flat, proximal end of the barbule, a group of refractile bodies appearing somewhat like colorless granules could be observed (Fig. 9). These might correspond to the colorless refractile bodies described by Hamilton (1940) and others, as the first stage in the formation of pigment granules within the melanophores, or they might be artifacts produced by the structural arrangement of the feather. Harman and Case (1941) have also observed colorless granules in the hair of guinea pigs.

The fact that pigment occurs in white feathers is not surprising, since gray ticking or black flecking in standard bred recessive white breeds is quite common, despite vigorous negative selection. Lippincott (1921) found rod-shaped black pigment granules in the down barbules of White Plymouth Rock and White Wyandotte chicks. They did not occur in all individuals, but in some cases they became sufficiently intense, in the Plymouth Rocks especially, to cause a ''smoky'' down coloration, a condition which is not known to affect the whiteness of the adult plumage. Furthermore it is hardly conceivable that apparently functional melanophores would be produced by white breeds, as reported by Hamilton (1940), and still not deposit any pigment whatsoever in the feather.

Contrary to these observations are those of Willier and Rawles (1940) who found no pigment in white varieties
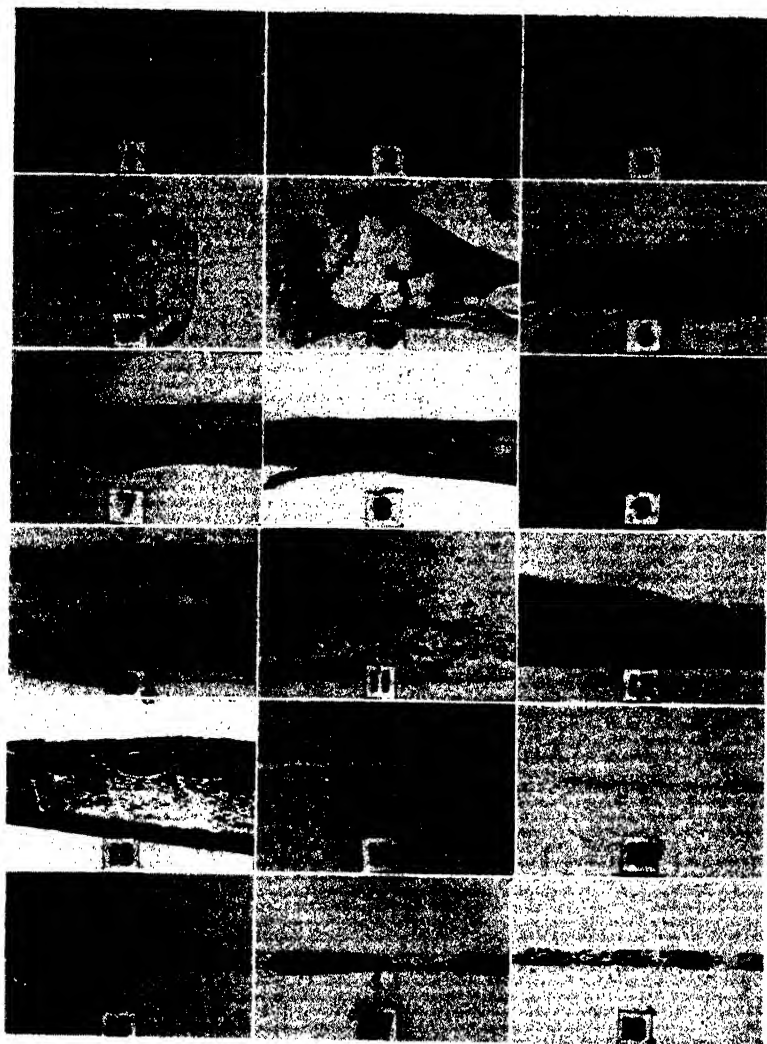
FIG. 1.   Black Minorca, free pigment granules.   Fig. 2. Blue Andalusian, free pigment granules.   Fig. 3. Rhode Island Red, free pigment granules. Fig. 4. Buff Plymouth Rock, cross-section of barb.   Fig. 5. Rhode Island Red, cross-section of barb.   Fig. 6. Buff Minorca, distal web barbule.   Fig. 7. Red Leghorn, distal web barbule.   Fig. 8. Black Australorp, distal web barbule.   Fig. 9. White Plymouth Rock, proximal web barbule.   Fig. 10. Buff Minorca, proximal web barbule.   Fig. 11. Blue Andalusian, proximal web barbule.   Fig. 12. Red Leghorn, proximal web barbule.   Fig. 13. Black Australorp, proximal web barbule.   Fig. 14. White Plymouth Rock, fluff barbule. Fig. 15. Buff Minorca, fluff barbule.   Fig. 16. Blue Andalusian, fluff barbule. Fig. 17. Rhode Island Red, fluff barbule.   Fig. 18. Black Minorca, fluff barbule.

of the Wyandotte, Plymouth Rock and Silkie breeds as
well as the Leghorn. They examined only fluff barbules
where the writers found pigment less frequently than
elsewhere in the plumage. They state that "usually" a
complete absence of pigment granules exists, indicating
that they may have found pigment in some cases.

Black Varieties

Black varieties examined included those of the Orping-
ton, Australorp, Leghorn, Cochin, Ancona (Mottled),
Minorca, and Andalusian breeds and black crossbreds
showing gold in the hackle (Table 1). In all of these
black specimens the granule shape and the distribution
of pigment within the feather parts appeared to be the
same. Any differences observed could be explained en-
tirely on the basis of variation in concentration.

In the fluff barbules very uniform rod-shaped granules
appeared (Fig. 18). These measured about 0.5 to 0.6 μ
by 1.0 to 1.3 μ and were distributed in bead-like rows run-
ning longitudinally in the barbule. Only in the proximal
region, near the attachment to the barb, was the arrange-
ment disorganized. If only a small amount of pigment
was present, the granules were localized in the distal
half of the internode. As the density of pigmentation
increased, the granules extended proportionally more
toward the proximal end. Even in quite densely pig-
mented specimens, however, the concentration of pigment
just proximal to the node could be observed. Usually
the last places in the fluff barbule to show pigment were
the nodes themselves. A marked dilution of the pigment
in the nodes was evident in all cases except where ex-
tremely heavy general pigmentation was found in the bar-
bule. This dilution at the nodes and the tendency to
clump just proximal to the nodes gave such fluff barbules
a distinctly segmented appearance when viewed under
low power magnification.

Both the hooked and curved web barbules (Figs. 8, 13)
were so densely pigmented that determination of granule

form was very difficult. The hooked barbules were uniformly and densely pigmented; the region distal to the hooks, however, appeared darker, probably due to the narrower and thicker structure in the region. They were always more densely pigmented than the curved barbules. The pigment also extended into the barbicels but only rarely did it extend the full length to the tiny hooklets or hamulae. Proceeding distally in the barbicels, the number of granules rapidly thinned until individual granules could be found and measured. The measurements here agreed with those in the fluff, the granules being characteristic rods about 0.5 μ in diameter by 1.3 μ in length, and invariably oriented longitudinally. In both hooked and curved barbules where pigmentation was very heavy, no cell boundaries or nuclei were seen. As pigmentation decreased, however, the granules were not deposited so close to the cell walls and pigment free areas indicated such boundaries. The nuclei of the original cells also became increasingly visible as the amount of pigment was reduced. Here the granules were densely applied to the nuclear wall, which gave the nuclei a distinctly oval appearance when viewed from the side. Near the cell boundaries rod-shaped granules could be observed, but elsewhere the barbules presented a ''pebbly'' appearance which some workers have interpreted as indicating round granules.

Acid extracted granules (Fig. 1) showed the same size and variation as those found within the feather. That no pigment had been lost during the process was indicated by the lack of any dissolved pigment in the filtrate from the acid hydrolysate. A light buff diffuse coloration was observed where the granules were concentrated within the feather. Although no color appeared in the filtrate from the acid hydrolysate, a similar effect as that seen in the feathers was observed wherever the granules tended to clump together in the acid insoluble residue. This is evidence that here at least, diffuse pigment as described by many earlier workers was not present.

Cross sections of the web from black feathers showed the pigment in the cortex of the barb to be distinctly stratified. These granules appeared round, as would be expected since the rods are placed longitudinally in the barb and a cross section of the rods was viewed. In the walls of the medullary cells, granules were also present and where a longitudinal plane was obtained they appeared rod-shaped. Pigmentation of the medulla was in no case observed inside of a cell cavity, but was always found within the keratinous walls of these cells. The barbules in such preparations showed no medullary cavities and the granules exhibited a uniform slightly oval appearance resulting from a diagonal section of the rod-shaped granules.

Willier and Rawles (1940) as well as Hamilton (1940) found black breeds of poultry to have rod-shaped pigment granules in the feathers. The latter described the melanophores of Jersey Black Giants as containing "long, thin rods with truncate ends" which were different from the short blunt rods of the Black Silkie melanophores. Lippincott (1921), however, found the rod-shaped granules of Black Andalusian, Orpington, and Langshan feathers to be identical.

Lloyd-Jones (1915) found two types of granules present in black pigeon feathers. One was a black rod, longer, more slender, and more variable in length than those found in the chicken. The other type was a sphere about the same size as that found in these studies in blue chicken feathers but which was not found in any black breed of poultry.

Blue Varieties

Blue Andalusians, Blue Cochins, Blue Splashed Andalusians and several blue crossbreds were utilized for the specimens of blue plumage (Table 1). In all cases where a blue color was exhibited, the granules were spherical and measured about 0.5 μ in diameter. In areas that appeared black, typical black rods occurred.

In the fluff barbules (Fig. 16) the spherical granules were usually found in bead-like rows oriented longitudinally, but frequently single granules could be found, and all were largely restricted to the distal half of the internode. In the most proximal area of the barbule the granules were more irregularly oriented and numerous spherical granules could be observed and measured. The Blue Cochin feathers were of a somewhat darker shade than the other breeds and showed a slightly more extensive pigment distribution, but no rod-like granules were found in the fluff.

The web barbules of the blues were unique in several ways. The granules were definitely spherical and appeared to be the same size as those in the fluff. Barbules from the periphery of the feather, however, showed typical black rods with the distribution typical of black granules. This accounts for the black lacing around the well marked blue feathers. The pigment was absent in the distal end of both kinds of barbules, the extent of the area devoid of pigment depending upon the shade of blue of the feather sample. The dark Blue Cochin barbules were virtually pigment free in the distal third, with only a few scattered granules remaining, while the lighter Andalusian had the pigment restricted to only the proximal third of the barbule (Fig. 11). Within the pigmented cells themselves, the pigment was restricted as compared with that in the black barbules. The positions of the nuclei of the cells were well defined due to a dense layer of pigment surrounding them. The other granules in the cell were grouped irregularly around the nucleus in the bead-like rows seemingly characteristic of round pigment granules. This left a relatively wide clear area between clumps of granules, but the cell wall was not well defined, since a few scattered granules or an occasional beaded string of granules extended entirely through the area. Nevertheless, a distinct cellular or segmental arrangement was noticeable even in the dark barbules of the Cochin.

Contrary to the situation in black feathers, the proximal barbules of blue feathers contained the most pigment. Here were found both a greater concentration of pigment within the cells and broader distribution of the granules. It would thus appear that the blue color of poultry is due to the combined effect of the shape of the granules and their restricted distribution within the individual cells, as well as in the barbule as a whole.

The residue from acid hydrolysis (Fig. 2) showed the same round granules seen in the feather and a few rod-shaped granules were found in the Andalusian, probably coming from the peripheral black barbules of the feather. More of these were found in the residue of Blue Cochin feathers where minute areas of black occurred in the web, giving the feather a stippled appearance.

These results are in complete accord with the earlier observations of Lippincott (1918) on blue chicken feathers. In pigeons, however, the situation seems to be somewhat different. According to Lloyd-Jones (1915) the granules although round, were two or more times the size of those found in the chicken. Also, in the pigeon barbs, the pigment was restricted to the medullary cells, the cortex being devoid of granules, while in the chicken, Lippincott (1918) described the uniform distribution of granules in both the cortex and the medullary cell walls.

Red Varieties

Feathers from Red Leghorns, New Hampshires, Rhode Island Reds, and Speckled Sussex were examined in studying the red pigment (Table 1). In red areas small spherical granules about 0.5 μ in diameter and of very uniform size were found. Another type of granule somewhat oval in character and measuring about 0.7 μ in diameter by 1.0 μ in length was found. The latter were far less numerous than the round granules and correspond in size, shape, and distribution to the granules found in buff feathers. In regions showing black pigmentation, only typical black rod-shaped granules were found. Their

distribution was also characteristic of black varieties but the pigmentation was more dense than normally found in true black breeds. In the transitional areas both round and rod-like granules were observable. Ladebeck (1921), Willier and Rawles (1940) and others, have described round and rod-shaped granules in the Rhode Island Red. No reference to the presence of oval-shaped granules among the round granules has been made by anyone other than Ladebeck, who considered them to be a variation from the black granules. The red pigment of pigeons is also present as spherical granules according to Lloyd-Jones (1915), but they are somewhat smaller being about 0.3 µ in diameter.

In the fluff barbules, the pigment was distributed throughout the internodes (Fig. 17). A tendency for the granules to be concentrated in the distal half of the node, and to be diluted or absent within the nodes, as was found in other colors, was also observed here. As the amount of pigment increased the light nodal areas tended to be obliterated except in the heavily pigmented fluff of the main tail feathers where the almost pigment free nodes provided a sharp contrast to the densely pigmented internodes. Red areas showed both the small round granules and the larger oval-shaped granules. Where smut occurred in otherwise red fluff, or in the gray fluff of the Sussex, the granules were rod-shaped. The small round granules were densely distributed throughout the internodes in bead-like, longitudinally oriented rows. The oval granules where discernible, seemed to be localized in the region immediately proximal to the node, the point at which the pigment normally shows the greatest concentration.

In the web barbules (Figs. 7, 12) the distribution of granules was similar to that in black feathers. Granules were of the spherical and oval types and were distributed generally to all structures except the tips of the hooks. Here again the hooked or distal barbules were more densely pigmented than the curved or proximal ones.

Pigmentation was also more dense in the proximal part of the barbules and when reduction in the amount of pigment was found, the loss occurred first in the barbicels, then the most distal portion of the barbule and receded proximally. In cases of reduced red pigmentation as found in New Hampshires or crosses between buff and red varieties, the loss seemed to be confined to the small round granules. The oval granules even seemed to increase in number, but this may have been due to the fact that some of them were masked by the round granules in the more densely pigmented feathers.

Determining the orientation of the densely distributed granules in the web was difficult, but here again it appeared that the round granules formed bead-like rows running longitudinally. The rows tended to be disorganized in the region of attachment to the barbs, and to curve around the position of the former cell nucleus, making its outline clearly distinguishable. They also tended to be absent from the area of the cell boundaries. This restriction was not so clean cut as was true in the case of black feathers since rows of granules frequently invaded the area and many single granules were scattered through it. Although apparently as densely distributed within the barbules as in the case of black feathers, they never became closely enough packed to present an opaque appearance such as was frequently found when black pigment was present. This would indicate that the density of color of the individual red granules is much less than that of blacks and that they therefore must be qualitatively different.

Residue from acid hydrolysis (Fig. 3) could not be expected to contain all of the pigment granules found in the feather sample, since some of the pigment was dissolved by the acid and appeared in the filtrate. However, a large number of the 0.5 μ spheres were found as were black granules, the number of the latter depending upon the presence and amount of black in the sample. They appeared exactly like those in the feather and it is un-

likely that the acid had affected them in any way. Notice-
ably absent from these preparations were the oval-shaped
granules although a few occurred if a large number of
feathers were treated for a short time with a small
amount of acid. Apparently, therefore, the oval-shaped
granules are the component which gives the coloring to
the acid filtrate of red feathers. Large amounts of the
so called "diffuse pigment" were observable in the
barbules wherever a quantity of pigment was present.
Since some of the red feather pigment was soluble in acid
it was impossible to determine whether diffuse pigment
as such was present in the feather, as contended by some
workers. However, since the reduction in the number of
oval granules could be correlated with the color of the
filtrate, it seems unlikely that diffuse pigment, as such,
was present. Furthermore, the same apparent diffusion
of pigment occurred in the residue wherever the granules
were clumped together as found in the feather.

Examinations of cross sections (Fig. 5) showed the
barb to contain much pigment in both the cortex and in
the walls of the medullary cells. The granules were in-
variably round as was expected and in the cortex showed
a distinctly stratified orientation. In the barbules, how-
ever, no definite arrangement was determinable.

Buff Varieties

Buff feathers from five breeds, Minorca, Leghorn,
Plymouth Rock, Cochin and Orpington were observed
(Table 1). The fluff barbules (Fig. 15) contained slightly
oval granules about 0.7 µ in diameter and 1.0 µ long.
These were placed in irregular rows but did not display
the bead-like arrangement exhibited by the round gran-
ules of blues and reds. Such granules were confined for
the most part to the distal half of the internode usually
with a definite clumping proximal to the node. Where
the granules were greatly concentrated, they extended
the full length of the internode and sometimes were even
found passing through the node.

In the web barbules the granules were not so numerous as in the fluff but retained their oval shape. They were more concentrated in the distal hooked barbules (Fig. 6) than in the proximal ones (Fig. 10) and although exter ·
ing the full length of both types of barbules they v more concentrated in the proximal portions. In cor
to other cases of reduced pigmentation, such as exl
by blue and light red feathers, a few granules wer/
in the barbicels. No definite arrangement into y/
observed as found in the fluff, most of the grany/
ring as individuals. The lighter the shade q/
fewer granules found and the more restrict⟨/
tribution. A light buffish cast impregnated in   ⋅ne
barbules in the area surrounding the granules, being more intense near the greater concentrations of granules and fading to almost white at a distance. Some workers have described this as ''diffuse'' pigment and such a claim is difficult to disprove in buff varieties due, as in the reds, to the extreme solubility of the pigment in acid. However, this appearance could be explained as the result of refraction of light from the pigment granules, causing the surrounding keratin to acquire a shade corresponding to the pigment in the granules.

The granules from buff feathers were completely soluble in acid and none could be recovered in the residue after treating for two hours. However, by boiling a large number of feathers for a short time in a small amount of acid as was done with the reds, a few granules were obtained. These varied from normal sized oval granules to spheres of very small diameter. Due to the ready solubility of this pigment, it was difficult to determine whether the smaller granules normally occurred in the feather. Their presence in such preparations, however, was probably due to the partial dissolution of normal sized oval granules. It is possible to say definitely from this procedure that the oval-shaped bodies described in the feather were actually pigment granules and not artifacts.

Cross-sections of the web showed round appearing granules in both barb and barbules (Fig. 4). The barbules were heavily pigmented in comparison to the barbs. In the cortex of the latter the granules exhibited a stratified arrangement similar to the colors previously described, but were much more scattered. In the medullary cell walls, granules appeared round if viewed on end but definitely oval if a longitudinal plane was observed.

Ladebeck (1921) described a very finely granular to diffuse pigment of a yellow to yellowish brown color as occurring in the buff breed which he examined. Danforth (1937) found that the melanophores of Buff Leghorns contained granules smaller and more nearly spherical than blacks. These had a light yellow color and were somewhat soluble in fixing agents. The latter description, although indefinite, seems to fit the rather large granules described in this investigation. In pigeons the granules are so fine that Lloyd-Jones (1915) was unable to determine their size or shape. They occurred in formless clumps or agglomerations and he considered buff as merely an attenuated form of red. Such a condition evidently does not exist in chickens even on the basis of the description of Danforth where granules may be assumed to have a measurable size.

Silver Patterns

As examples of silver colored breeds, Columbian Plymouth Rocks, Light Sussex, and Light Brahmas were included (Table 1). The standard color description for females of this color pattern specifies black striping in the hackle and tail coverts and black in the main tail and wing feathers, the rest of the body feathers being white with "light bluish slate" undercolor.

The Columbian Plymouth Rock feathers examined were excellently marked, with a sharp line of demarcation between the white web and the gray fluff, the latter fading slightly toward the proximal end. The fluff barbules of this breed showed remarkably uniform rod-shaped gran-

ules of about 0.5 to 0.7 μ diameter and 1.0 to 1.3 μ length. These were concentrated just proximal to the nodes but were also found distributed somewhat less densely throughout the rest of the internodes and the nodes as well. The granules were oriented longitudinally to the barbule and no exceptions to this arrangement were observed. Wherever the granules were concentrated, as near the nodes, a yellowish brown shading appeared which might be considered as diffuse pigment by some workers. Since this appearance was not observed about any single granule it is doubtful that a diffuse pigment as such is present. It is more likely that such an effect is produced by the refraction of light by the grouped pigment granules. Furthermore, no pigment was observed in the filtrate from acid hydrolysis of such feathers, and in microscopic preparations of the residue, wherever the granules tended to clump together, an effect was produced as if diffuse pigment were present. If diffuse pigment were present as such, it would be expected to appear in the filtrate and not in the residue. Approximately the same density of pigmentation was found in the web barbules as was found in the average recessive white but instead of being concentrated at the base, the pigment was distributed throughout the entire length, the distal barbules containing noticeably more granules than the proximal ones. Occasionally granules were found in the barbicels (hooks) themselves, even though there might be none for some distance about them. The granules varied from rod-shaped, as in the fluff, to spherical granules varying from 0.5 μ in diameter down to the limits of visibility. In the slides of acid hydrolysis residue, however, the granules appeared to be almost entirely rod-shaped. This may be explained on the basis of the observation of Hamilton (1941) and Danforth (1937) that the shape of the granules appears to be changed after ingestion into the cells. In the present case, however, it may be that the apparent change in shape is due to partial masking by the keratin substance.

The Light Brahma and Light Sussex feathers were much too light in undercolor according to standard description, the gray being confined almost entirely to the rachis. The web barbules here were identical in pigmentation to those of the Columbian Plymouth Rock, although somewhat fewer granules were present. In the fluff barbules, however, the rod-shaped granules when present were concentrated in the distal third of the internodes with no pigment in the intervening spaces. As the amount of pigment increased, granules extended more proximally into the internodes, the nodes themselves being the last to be pigmented.

Other silver patterns examined included Silver Campines, Silver Penciled Plymouth Rocks and Barred Plymouth Rocks (Table 1). In the two former breeds, the granules and their distribution in both fluff and web barbs were typical of self (solid) blacks. In preparations of acid residues the granules were also characteristic rods as found in true blacks. In the fluff barbules of these varieties a lower concentration of pigment seemed to be necessary for the granules to pigment the nodes to an equal density as the internodes. In the white bands of the Campine the pigment was progressively reduced in each distally succeeding barbule. This was accomplished by eliminating pigmentation from the distal ends until a minimum in the barbules centrally located in the bands was reached. Then the proximal pigmentation gradually increased toward the next dark band. A few rod-shaped granules remained in all of the white barbules, and the light area could not be differentiated from barbules of white feathers. In the Silver Penciled Plymouth Rock, however, a large quantity of pigment remained in the distal half of the hooked barbules of the white area, while the curved barbules became almost colorless. The presence of so much pigment gave the penciled area of the sample feather a light dun appearance.

The granules of the Barred Plymouth Rock were slightly shorter on the average and somewhat more vari-

able in size than were those of normal blacks. Willier and Rawles (1940) observed this same characteristic although it seems to have been overlooked by Lippincott

TABLE 1

A SURVEY OF CHICKEN FEATHER PIGMENTS

Breeds	Apparent color of cushion feathers	Granule shape	Average granule size in microns	Solubility		Slope of log. E curve 500 to 700 millimicrons (× 100)
				Conc. HCl	0.5N NaOH	
Black Minorca	Black	Rods	0.5 × 1.3	−	+	− 0.3303
Black Orpington	Black	Rods	.5 × 1.3	−	+	− .3107
Black Australorp	Black	Rods	.5 × 1.3	−	+	− .3526
Black Cochin	Black	Rods	.5 × 1.3	−	+	− .3391
Black Leghorn	Black	Rods	.5 × 1.3	−	+	− .3335
Black Andalusian	Black	Rods	.5 × 1.3	−	+	− .3391
Barred Plymouth Rock	Black & White	Rods	0.5 × 1.0	−	+	− 0.3470
Columbian Plym. Rock	Black & White	Rods	.5 × 1.3	−	+	− .3224
Silver Campine	Black & White	Rods	.5 × 1.3	−	+	− .3224
Sil. P. Plym. Rock	Black & White	Rods	.5 × 1.3	−	+	− .3019
Ancona	Black & White	Rods	.5 × 1.3	−	+	− .3372
Blue Andalusian	Blue & Black	Spheres Rods	0.5 .5 × 1.3	−	+	− 0.3706
Blue Cochin	Blue & Black	Spheres Rods	.5 .5 × 1.3	−	+	− .3036
Gold Campine	Black & Buff	Rods Ovals	.5 × 1.3 .7 × 1.0	+	++	− .3497
Speckled Sussex	Red & Black	Rods Spheres	.5 × 1.3 .5	++	+++	− .5003
Brown Leghorn	Black & Brown	Varies		−	+	− .3487
Partridge Plym. Rock	Black & Red	Rods Ovals Spheres	.5 × 1.3 .7 × 1.0 .5	+	++	− .3401
Rhode Island Red Main Tail	Black & Red	Rods Spheres	.5 × 1.3 .5	++	+++	− .4763
Rhode Island Red	Red	Spheres Ovals	.5 .7 × 1.0	++	++++	− .6302
Red Leghorn	Red	Spheres Ovals	.5 .7 × 1.0	++	++++	− .6402
Buff Orpington	Buff	Ovals	0.7 × 1.0	+	+++	− 0.4481
Buff Minorca	Buff	Ovals	.7 × 1.0	+	+++	− .6198
Buff Cochin	Buff	Ovals	.7 × 1.0	+	+++	− .4089
Buff Plymouth Rock	Buff	Ovals	.7 × 1.0	+	+++	− .5406
Buff Leghorn	Buff	Ovals	.7 × 1.0	+	+++	− .5331
White Wyandotte	White	Varied		−		− 0.2518
White Langshan	White	Varied		−		− .3242
White Silkie	White	Varied		−		− .2620
White Plymouth Rock	White	Varied		−		− .3179
White Minorca	White	Varied		−		− .3372
White Leghorn	White	Varied		−		− .2749
White Orpington	White	Varied		−		− .2518

(1918). The average granule size was about 0.5 μ by 1.0 μ. They did not vary much in diameter but varied considerably in length, occasional granules being as long

as normal black rods while a few others closely approached spherical proportions. Lippincott observed spherical granules only rarely in this variety. The concentration of pigment in the feather was in all cases more sparse than in black breeds. At the proximal edge of the white bar, the pigment free area began at the tip of the barbules and increased in successive barbules. In the middle of the white bar, only a few scattered granules were found in the barbules. If the bar was narrow, black pigment was evident in the tips of the barbules even before the proximal pigment had been entirely eliminated. More of the distal region of each succeeding barbule was pigmented until the completely pigmented barbules of the black band were reached. Thus no barbule was ever entirely devoid of pigment in both the tip and base at the same time, and the pattern of the bar on the individual barb was V-shaped. The light barbules of all three varieties were identical to those from white feathers and possessed many of the so-called colorless granules, some even occurring in the barbicels.

Gold Patterns

Gold color patterns examined included Gold Campine, Partridge Plymouth Rock and Brown Leghorn varieties (Table 1). In the Plymouth Rock and Campine the black areas had rod-shaped granules again typically black. The distribution of the rods was also typical of that in black breeds except that, as in the silver patterns, the fluff barbules required a lesser concentration of granules in order for the nodes to be pigmented. Nevertheless a distinctly segmental arrangement was maintained in all but the darkest fluff barbules. The light bars of the Campine feathers showed a buff coloration and the barbules appeared microscopically similar to those of buff feathers. The light bands of the Partridge Rock were more heavily pigmented and looked more like red pigmentation. However, the intense pigmentation of these barbules made it very difficult to determine whether a dilute

black or a red color was present. The fact that both feathers contained an acid soluble pigment indicated that some pigment other than black was present. Moreover, the acid hydrolysis residue of the Gold Campine feathers contained only black rods indicating that the oval-shaped buff granules must have gone into solution. The residue of Partridge Rock feathers, on the other hand, contained both typical black rods and round granules typical of red feathers.

The Brown Leghorn cushion feathers were unique in that the granules showed a great variation in size, ranging from rods about 1.5 μ long by 0.5 μ in diameter which are longer than black granules, down to almost spherical forms about 0.5 μ in diameter. The average of these lengths, however, was somewhat less than that of typical black rods. These variations were quite evident both in the acid residue and in the fluff barbules but none of these granules were acid soluble, indicating that probably no chemical difference exists between the different types. Whether the granules of the red areas of other regions of the plumage show different characteristics was not determined. Due to the density of the pigmentation and compact arrangement in the web barbules it was not determined definitely whether granules of a special size or shape were localized in the lighter-colored barbules. These lighter brown areas appeared, under the microscope, identical to the darker regions except that they were less concentrated.

Ladebeck (1921) found the feathers of the Brown Leghorn to contain variously shaped granules. He believed that all transitional forms from black to red were present. Furthermore, he believed that the intermediate color between black and red, such as found in the lighter areas of the cushion, consisted of granules which were transitional forms or ellipsoidal in shape. Although this situation might conceivably exist, it was not possible to demonstrate the fact by the techniques employed in this study, nor was the evidence sufficient to prove otherwise.

Discussion

Ladebeck (1921) stated that the distal barbules are more heavily pigmented than the proximal ones but he did not study the Blue Andalusian in which Lippincott (1918) found the reverse situation. The present investigation fully corroborates the observations of both authors. In the more extensive survey of chicken feather colors here recorded, in all colors except blue the distal barbules contained the most pigment. Lloyd-Jones (1915) described the distal barbules as uniformly containing the heaviest pigmentation in all colors of the pigeon.

In all cases where round granules were observed, they tended to be deposited in bead-like rows longitudinally oriented either in the barbule or barb. Black rods or oval-shaped granules were also uniformly arranged parallel to the long axis of the feather structure in which they were found. Only in a short region adjoining the barb was this precise organization varied, and here the arrangement was very irregular. These observations again confirm and extend the earlier work of Ladebeck (1921).

In the fluff barbules of all colors the distribution of pigment granules seemed to depend upon the density of the pigmentation. Where little pigmentation was present the granules were concentrated within a very limited area in the distal end of the internode. This suggests that the first pigment is laid down at this location in these barbules. Increases in the amount of pigment up to a certain point seem to result in greater concentration at this locus without any notable distribution to other areas. Further increments of granules result in pigment extending more and more proximally within the internode. In most cases a very dense pigmentation of the internodes was necessary before the pigment concentration in the distal portion was obliterated from view or granules were deposited within the nodes, which were the last regions to be pigmented. The required density within

the internodes which allowed the nodes to become pigmented, however, seemed to vary among varieties and among sections on the same birds. In Rhode Island Reds the fluff of the main tail feathers showed extremely dense pigmentation and the nodes were almost completely pigment free. In body feather fluff of the same bird a much lower concentration was required to allow the nodes to be equally as densely pigmented as the internodes. Also in Silver and Gold Campines and other breeds having gold and silver patterns, the required density was not so great as it was in the blacks. Nevertheless, the general rule as explained above seemed to hold for all colors.

Diffuse pigment has been described by many workers as present in animals. Included among these are Haecker (1890) who observed diffuse pigment in red and yellow bird feathers, and Ladebeck (1921) who described it as present in a yellow variety of poultry. Other workers have staunchly denounced the idea of diffuse pigment. As pointed out by Meirowsky (1912) and others later, the so-called diffuse pigment does not occur except when granules are present. Esskuchen (1927) found diffuse pigment associated with black and red cattle pigments, more with the latter and less with the former. Harman and Case (1941) described red granules of guinea pig hair as bright and translucent and associated with a large amount of brilliant, orange-red diffuse pigment. Chocolate granules were darker and denser; and less diffuse pigment, of a reddish hue, was found with them. The black granules were the most dense and were associated with the least amount of diffuse pigment, that present being a faint yellowish color. This situation might be explained by assuming that the diffuse pigment was a degradation product of the granules and as a result of this "chemical or physical reaction" with the keratin, the pigment of the granules diffuses into the keratin, leaving the granule more translucent and less dense than formerly. The above authors seemed to favor this hypothesis. That such a hypothesis is untenable, in the case of

chicken feathers at least, is evident from the fact that Hamilton (1940, 1941) and Danforth (1937) and others have observed that the definitive refractive nature of the granules in the active melanophores has been determined before they come into contact with the keratin of the feather.   However, a relationship similar to that described for the guinea pig by Harman and Case exists between the granular and apparently diffuse pigments of the chicken feathers, and therefore demands an explanation.   The black feather granules were associated with a slight yellowish appearing diffuse coloration, the yellow feather granules with a more widely spreading diffuse coloration, and the red feather granules with a much deeper colored, apparently diffuse substance.   It was further noticed that in no case did individual black granules show this phenomenon, its appearance being associated only with groups or clumps of granules.   The same situation only to a lesser degree was observed in red feathers, *i.e.*, fewer granules in one group were required for the appearance of the so-called bright red diffuse pigment.   Just what the result would be if red pigment granules were as sparsely distributed as the granules of buff feathers is a matter of speculation only, as no such condition has been found.   The granules of buff feathers were never sufficiently concentrated to form definite clumps, nevertheless, a yellowish cast in the keratin extended outward for some distance from only small groups of granules, becoming gradually lighter until the keratin again became colorless.   On acid hydrolysis the feathers from, black, blue, and silver colored birds showed no coloration of the filtrate, indicating that an acid soluble diffuse pigment as described by Gortner (1911) was not present.   Moreover, in the insoluble residue remaining after two hours of boiling in concentrated HCl, the same diffuse yellowish color was present wherever the black granules were clumped together as in the feather.   Although an acid soluble pigment was found in red and buff feathers, its appearance in the acid filtrate has been asso-

ciated with the disappearance of the oval-shaped granules characteristic of these two types of feathers. Furthermore, in the insoluble residue of red feathers, this phenomenon also occurred wherever even a few of the round red granules are clumped together. It is improbable that a pigment diffused in the keratin could withstand two hours in boiling HCl, much less still fail to pass through ordinary filter paper. The absence of sufficient buff granules at any one locus immediately rules out the possibility that this phenomenon might be due to the presence of many granules which are out of focus, although this may accentuate the condition in some colors. Therefore, it would appear that only one tenable hypothesis remains for the explanation of this phenomenon in chicken feathers. Differences in size, degree of translucence and the color of the granules would cause them to have different optical properties. Therefore, the passage of light through a granule or the reflection of light from its surface would result in a diffusion of color throughout the adjacent keratin, the amount depending upon the density of the granules and the color dependent upon the color of the granules.

Willier and Rawles (1940) described the shape of the pigment granules as breed specific. Their study, however, considered only two types having definitely black pigmentation, the Black Minorca and the F_1 females from Barred Plymouth Rock females by Rhode Island Red males. They did not describe the differences found between the granules from these specimens. Hamilton (1940) was more explicit. He described the granules in cultured melanophores of Jersey Black Giants as ''long, thin rods with truncate ends'' in contrast to the short blunt rods of the Black Silkie bantam. Unfortunately no Jersey Black Giant feathers were available for checking this observation by the methods employed in this study. The term ''breed'' has also been used quite loosely by these workers. Hamilton includes the guinea with a list of poultry breeds. These workers, moreover,

described differences between the granules of red and black feathers and referred to these types as breed specific, even though varieties exhibiting either or both of these types of granules may be found within a single breed of poultry. On the other hand Lippincott (1921) found the rod-like granules of three black breeds to be identical. In the present study no apparent differences were found between the granules of any of the black types, even at a magnification of 1900 diameters. A similar condition also held true within all red, buff and blue varieties studied. It would appear, therefore, that the evidence favors a color or genotypic specificity of granule shape rather than a breed specificity.

CHEMICAL STUDIES OF FEATHER PIGMENTS
Materials and Methods

The feathers analyzed in this phase of the study were from the same samples as those utilized for the histological examinations of the previous section (Table 1). The observations on solubility were incidental to the preparation of acid residue for microscopic examination, and the preparation of basic solutions for spectrophotometric analysis. Therefore, all observations pertain to the solubility in hot and cold 6N HCl and 0.5N NaOH.

For the spectrophotometric analyses, 0.2 g. feather samples were weighed out on an analytical balance. For the sake of a uniform treatment, the feathers in the present study were all digested for two hours in 100 cc. of 0.5N NaOH and then allowed to cool. After standing for 48 hours, the samples were brought to volume and filtered through a fritted glass filter. Hydrolysis in all cases was nearly complete, since never more than a trace of pigment was left in the filter. According to Daniel (1938) the slope of the log. E curve for mouse hair was not affected by length of boiling time although a very short boiling time resulted in only partial hydrolysis. The only effect of varying the boiling period was to shift the line up or down on the graph as a result of variations in concentration.

The optical density of each solution was then determined by means of a visual spectrophotometer at wave lengths of 500, 600, and 700 millimicrons, with the average of 10 readings at each position establishing the data points. The reading from the instrument ($\log \frac{I_0}{I}$) is referred to as the optical density. In order to place the samples on a quantitatively comparable basis the optical density was converted to a 1 per cent. concentration and a 1 mm depth of solution, the resulting figure being the extinction coefficient ($E \frac{1\%}{1 \text{ mm}}$). For graphical presentation, the $\log E \frac{1\%}{1 \text{ mm}}$ values were plotted against wave length, since this produced a straight line for the pigments studied, while plotting the E values resulted in a curve. If two pigments studied are of the same chemical and physical composition, their spectral curves should coincide when equalized for concentration by simple vertical displacement. Variation in the slope of the curves, however, indicates qualitative differences between the samples. A numerical expression of the curves was obtained by taking the difference between the $\log E \frac{1\%}{1 \text{ mm}}$ values at 500 and 600 millimicrons, the resulting figure being referred to as the slope × 100. Only these two readings (500 and 600 millimicrons) were utilized in arriving at this value because they are less subject to error than is the point at 700 millimicrons due to the very weak absorption of the solutions at the latter wave length.

Solubility of the Pigments in Acid and Alkali

The solubility of the feather pigments of different breeds of poultry are indicated in Table 1. The pigments of all the self black, black and white, and blue feathers were resistant to the action of 6N HCl, no traces of pigment being found in the filtrate after hydrolyzing for two hours. Surprisingly, the pigment of Brown Leg-

horn cushion feathers seemed to be as resistant to the acid as was that from black feathers, even though their appearance indicated the presence of a light-colored pigment.

The pigment of Gold Campine and Partridge Plymouth Rock feathers showing a small amount of buff and red respectively alternating with black bars, was slightly soluble in the acid. The residue of the former showed rod-shaped granules characteristic of black feathers, while that of the latter showed rod-shaped and also a few round granules. Pigment from the red feathers showed a greater solubility in acid than pigment from the black, the Gold Campine, or the Partridge Plymouth Rock feathers due to the presence of a greater amount of of soluble pigment. However, round granules in the residue, which were not dissolved by long boiling, were characteristic of all red feathers. In regions of the plumage where black color appeared in the feathers, rod-shaped granules also appeared in the residue. Buff feather pigments were entirely dissolved by the acid in a very short time. A few acid insoluble pigment granules of varying shapes were found in all of the white breeds, which fact is in agreement with the histological observations already presented.

The presence of oval-shaped granules in red feathers similar to those in buff feathers (Table 1), along with the loss of the oval granules from both colors on acid hydrolysis, and the retention of round granules by the red feather residue, seem to indicate that only one pigment, buff, is soluble in concentrated hydrochloric acid. If this be true, it would strongly suggest that the red feather is genetically the same as buff with an acid insoluble brown pigment superimposed upon it. Each has been found from genetical analysis to carry the sex-linked gold factor.

All the pigments were completely dissolved by 0.5N NaOH but the rate of hydrolysis varied markedly. The acid insoluble pigment of the black appearing feathers

was only slowly soluble in the alkali, requiring about 45 minutes to an hour of boiling before being noticeably dissolved. Solubility was determined by examination of the filtered solution. Buff pigment on the other hand was apparently completely dissolved by the time the boiling point was reached, or about 10 to 15 minutes. Red feather pigments appeared to be the most soluble since their pigment began diffusing through cold alkali immediately upon introduction into it.

Differences in the alkali solubility between the black and the red or yellow pigments of various animals have been reported by other workers. Spöttel (1914) and Lloyd-Jones (1915) found such a difference in pigeons, Einsele (1937) in mice, Russell (1939) in guinea pigs, and Ladebeck (1921) and Görnitz (1923) in chickens. Görnitz also found that the red feathers contained a slightly more alkali soluble pigment than did the buff, and that both of these pigments were more readily soluble than the black pigments. Since the solubility of black pigments is expedited by oxidation with hydrogen peroxide, Görnitz postulated that the naturally occurring red and yellow bird feather pigments were oxidation products of black pigment.

Spectrophotometric Analysis

The slopes of the log. E curves for all feather solutions analyzed are given in Table 1. The slopes for all of the feathers showing only black pigment are the same, averaging −0.33 with an extreme range of only 0.04. The curves for the blue feathers appear to be the same as those for black, the slope of −0.34 and range of 0.07 being very close to the values for black pigment.

Buff feather pigment curves are distinctly different from the blacks, having an average slope of −0.52 with a range of 0.22. The buff solutions were very dilute and were therefore difficult to read precisely, which may account for some of the variability of these samples. On the other hand, buff breeds frequently show small amounts

of black in the plumage which in such dilute solutions as these would have an appreciable effect on the slope of the curves.

The average slope of the curves for feathers from red breeds is – 0.63 with a range of 0.04. This slope is decidedly more steep than that of buff or black feathers, indicating that this color is chemically distinct from either of the others.

The flattest curves were obtained from feathers of white breeds and had an average slope of – 0.28. Due to the small amount of light absorbed by these samples, considerable relative variability would be introduced by traces of black pigment. That this was the case is suggested by the fact that the range of the slope for the log. E curves was only 0.09 which is less than that of the buff samples.

The differences in slope exhibited by the solutions of different colored feathers appear to be very consistent and decided. Zwicky and Almasy (1935), however, obtained absorption curves for black and red horse hair which were as widely different as were those from black and red feathers but nevertheless concluded that the two colors were not different entities. In order to demonstrate that the variations in these curves are indicative of chemical differences rather than chance, statistical analysis was applied to a group of data. For this statistical treatment three similar samples of Blue Andalusian feathers and five replicates from a representative breed of each of the four other self colors were used. The samples were first washed in ether in an effort to further reduce the variability, and to determine whether any impurities so removed had affected the shape of the curves. Such treatment, however, did not appreciably affect either factor. The slopes of the absorption curves (Table 2) were then determined as previously described. The curves obtained are shown graphically in Figure 19. The analysis of variance for these slopes (Table 4) yielded an F value of 159. Since the required value for

the 0.1 per cent. level is only 7.467, the probability of the five average slopes being from the same population is far less than one in a thousand. Further analysis
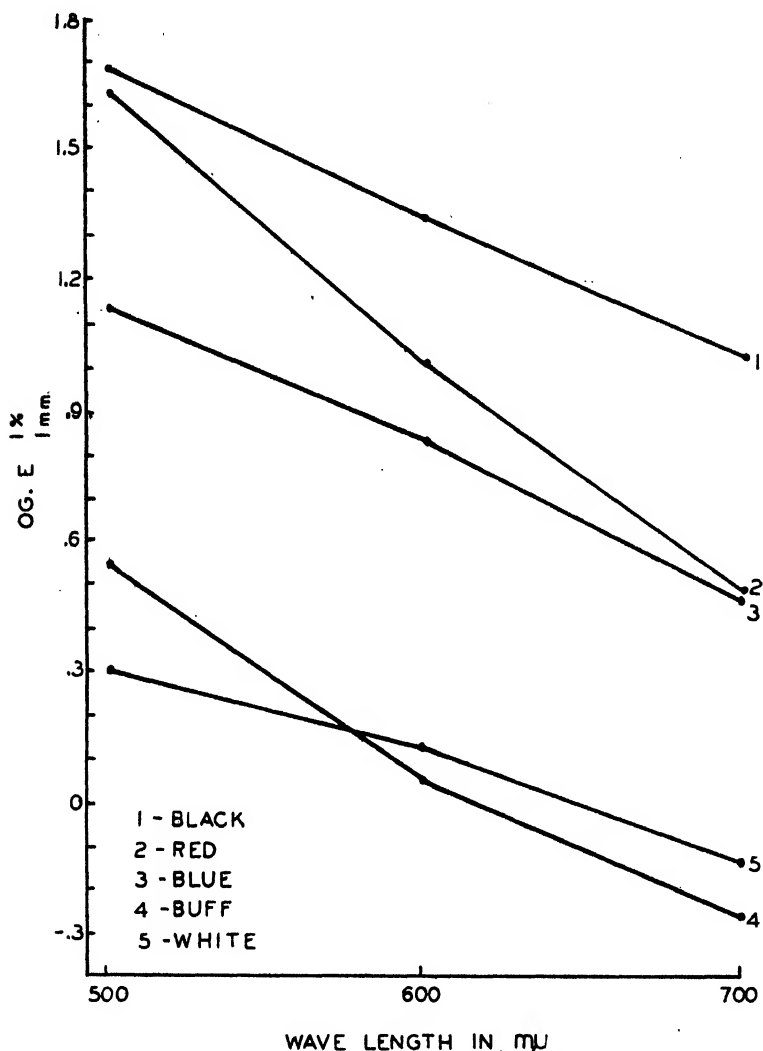
Fig. 19. Typical log. E curves for alkali solutions of various colored chicken feathers.

showed the least significant difference between any two averages at the 0.1 per cent. level of probability to be 0.0745. Since the difference between any pair of color

averages, except that between blue and black, is far greater than this figure, it is evident that the differences are statistically highly significant. The actual difference between the average slope of blue and black feather pigments is 0.003 well within the 50 per cent. level of probability, 0.0138, indicating that the two sampling values are probably from the same population.

Baker (1942) was able by similar treatment to demonstrate an equally great difference between black and red guinea pig hair pigments. However, Dunn and Einsele (1938) using the colorimeter and Daniel (1938) using the spectrophotometer to compare the absorptive power of various mouse pigments, concluded that the samples with which they worked varied only quantitatively. Their evidence would appear quite conclusive until it is noted that these workers used only genotypes of the C series or Agouti series and that no true reds and yellows produced by the genotype ee were examined. Gremmel (1939) also concluded that the black and red pigments of horse hair did not differ qualitatively. Examination of Gremmel's data, however, show that the colorimetric comparison of the different colored samples gave exactly the results to be expected if red pigment is qualitatively different from black pigment. It seems probable then, in spite of the conclusions of Zwicky and Almasy (1935), Daniel (1938), and others, to the contrary, that qualitative differences in the pigment of epidermal structures of animals do exist.

It is seen in Table 1 that the slope of the absorption curves of feathers containing both red and black pigment such as Gold Campine and Speckled Sussex are variable. The curves for the Gold Campine, Brown Leghorn, and Partridge Rock do not appear to differ from those of black feathers. The absence of an acid soluble pigment in the Brown Leghorn feathers may explain their close resemblance to black, but the same explanation would not hold for the other two since they both contain an acid soluble pigment. The similarity of the absorption curves

for feathers of these two breeds to those of black feathers may, however, be due to the proportion of the acid soluble pigment present being too small to affect the slope of the curve. When a larger proportion of the more soluble pigment is present as in Speckled Sussex body feathers and Rhode Island Red main tail feathers, the slope of the

TABLE 2

COMPARISON OF THE SLOPE (×100) OF THE LOG. E CURVES OF DIFFERENT COLORED FEATHERS. (ETHER WASHED SAMPLES)

Breed	Sample No.	Slope at 2 days (×100)	4. 5 months		7 months	
			Slope (×100)	Change from 2 days	Slope (×100)	Change from 2 days
Black Minorca	B. 1	− 0.3170	− 0.4660	− 0.1490	− 0.4389	− 0.1219
	B. 2	− .2801	− .4484	− .1083	− .4425	− .1624
	B. 3	− .3233	.....	.....	.....	.....
	B. 4	− .3028	− .4724	− .1696	− .4559	− .1531
	B. 5	− .3170	− .4895	− .1725	− .4522	− .1352
Average		− 0.3080*		− 0.1649		− 0.1431
Blue Andalusian	Bl. 1	− 0.3089	.....	.....	.....	.....
	Bl. 2	− .3036	.....	.....	.....	.....
	Bl. 3	− .3045	− 0.4855	− 0.1810	− 0.4510	− 0.1465
Average		− 0.3057*		− 0.1810		− 0.1465
Rhode Island Red	R. 1	− 0.6289	− 0.6990	− 0.0701	− 0.6326	− 0.0037
	R. 2	− .6635	− .7235	− .0600	− .7825	− .1190
	R. 3	− .6498	.....	.....	.....	.....
	R. 4	− .5935	− .8041	− .2106	− .8239	− .2304
	R. 5	− .5800	− .7570	− .1770	− .5901	− .0101
Average		− 0.6214*		− 0.1294		− 0.0908
Buff Orpington	Bu. 1	− 0.4274	− 0.6234	− 0.1960	− 0.4802	− 0.0528
	Bu. 2	− .5171	− .6716	− .1545	− .9508	− .4337
	Bu. 3	− .4461	− .6757	− .2296	− .5834	− .1373
	Bu. 4	− .4711	− .8013	− .3292	− .6460	− .1749
	Bu. 5	− .5544	.....	.....	.....	.....
Average		− 0.4922*		− 0.2273		− 0.1997
White Wyandotte	W. 1	− 0.2292	− 0.4776	− 0.2484	− 0.7213	− 0.4921
	W. 2	− .2240	.....	.....	− .0364	− .4973
	W. 3	− .1391	.....	.....	− .4191	− .2101
	W. 4	− .2090	− .7570	− .5480	.....	.....
	W. 5	− .1624	.....	.....	.....	.....
Average		− 0.1927*		− 0.3982		− 0.3998

* Least significant difference between any two means at 50% level = 0.015; at 0.1% level = 0.0863.

curves falls somewhat intermediate to those of black and red, closely approximating that of buff.

According to Beer's Law, if two different pigment solutions are combined one would expect the absorption of light at any given wave length expressed as E to be cumulative, and the curve for the mixed solution should fall between the curves for the pure solutions, the exact location depending upon the proportions in the mixture.

Therefore, proportional mixtures of red and black feathers were prepared and their absorption curves determined (Table 3). The purpose of this test was to determine whether it is possible to estimate accurately the proportion of red and black in unknown solutions. It is evident from Figure 20 that the curves fall in order of their proportion of the two pigments, and in Table 3 it
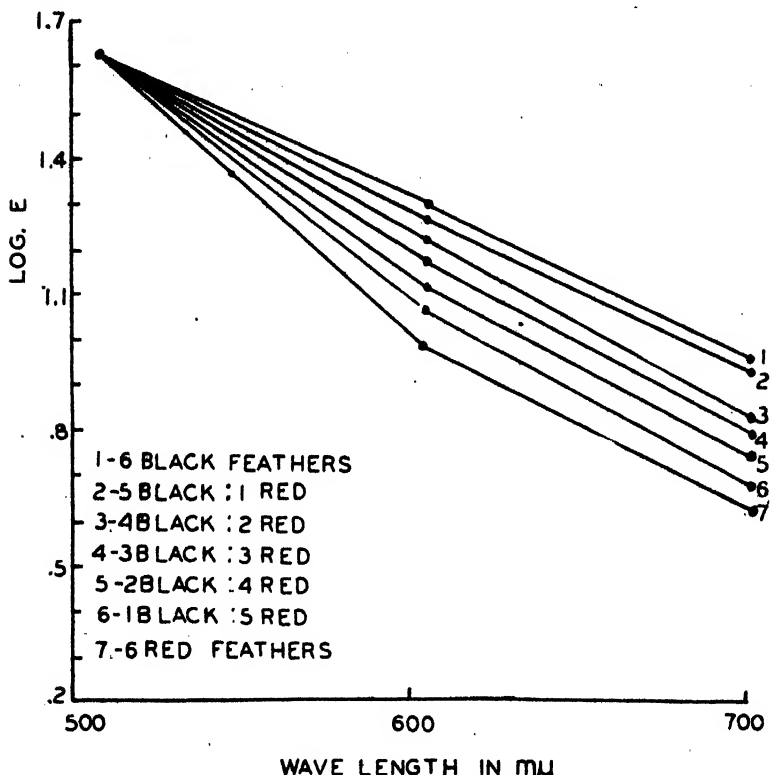
FIG. 20. Log. E curves for mixtures of alkali solutions of red and black chicken feathers, adjusted so that the values at 500 mμ coincide.

is shown that the curves of the mixtures fall almost proportionally (on the E scale) between those of the pure red and black feathers.

Zwicky and Almasy (1935) found that horse-hair solutions were not altered on long standing. However, Baker (1942) found that the slope of the absorption curves for solutions of black guinea pig hair changed from −0.22

TABLE 3

SLOPE OF SPECTROPHOTOMETER CURVES FROM 500–600 MILLIMICRONS FOR MIXTURES OF RED AND BLACK FEATHER SOLUTIONS

Proportion	Experimental Slope	Expected Slope
Red	− 0.629	
5R : 1B	− .545	− 0.559
4R : 2B	− .503	− .499
3R : 3B	− .443	− .445
2R : 4B	− .397	− .398
1R : 5B	− .348	− .356
Black	− .317	

to − 0.48 in a period of four months. Since this change in slope is in the direction of red, he advanced this fact as evidence that red pigment is an oxidized form of black. Table 2 shows the effect of standing for four and a half and seven months on the slope of the absorption curve of chicken feather pigments. It is immediately clear that at four and a half months a change has occurred, although in no instance, except in the case of buff, was the change of as great a magnitude as the 0.26 shift reported by Baker. However, it is also seen that white feathers, which contain very little or no pigment, but only keratin, have undergone an even greater change than any of the chicken pigments or the black guinea pig pigment. It is therefore obviously fallacious to reason that a steepening of the slope of the absorption curves for colored feathers or hair is indicative of a change in the nature of the pigment when pigmentless feathers show a greater change. Baker did not report the effect of standing on white hair solutions. This point of criticism derived from white feathers, however, can not hold against the results of Arnow (1938) and Baker (1942) when they compared artificial red melanin, prepared by oxidizing

TABLE 4

ANALYSIS OF VARIANCE OF DIFFERENT FEATHER PIGMENTS

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	22	0.5900	
Between color means	4	.5735	0.1434**
Within groups (error)	18	0.0165	0.0009

** Value of F = 159; value required for significance at the 0.1 per cent. level - 7.467.

black dopa melanin which should be relatively free of impurities, with natural red pigment. The absorption curves of the two red melanins were identical, indicating that it would be possible for red melanin to be an oxidation product of black.

An equilibrium seems to have been reached by the white feather solution at four and a half months, and at seven months it had not changed further. On the other hand the colored solutions at seven months had all shifted back slightly toward the original value.

SUMMARY AND CONCLUSIONS

Cushion feathers of 37 different breeds and varieties of poultry, consisting of reds, blues, blacks, buffs, whites and various gold and silver patterns were studied histologically and chemically.

Histological Studies

Black feathers or feather parts showed rod-shaped granules of only slight variability in size averaging about $0.5\,\mu$ by $1.3\,\mu$. The granules were insoluble in 6N HCl, but slowly soluble in 0.5N NaOH.

Feathers of blue varieties contained very uniform round granules about $0.5\,\mu$ in diameter, distinctly different in appearance from those of red feathers. These were acid insoluble but were slowly soluble in the alkali.

Both dominant and recessive white feathers showed a few black pigment granules of irregular size in both web and fluff barbules.

Buff feathers contained oval-shaped granules averaging about $0.7\,\mu$ in diameter by $1.0\,\mu$ in length. These granules were readily soluble in either HCl or NaOH.

Red feathers contained oval granules similar in size, shape, and solubility to those in buff feathers. Round granules of very uniform size about $0.5\,\mu$ in diameter, very soluble in 0.5N NaOH and insoluble in 6N HCl were also found.

In all specimens except those of blue varieties, the distal (hooked) barbules were more heavily pigmented than the proximal (curved) barbules.

In all cases where round granules were found, they tended to be deposited in bead-like rows longitudinally oriented in the feather structure. Rod- or oval-shaped granules were also oriented longitudinally in the feather, but were not in such compact rows as were the red granules. In a short region of the barbule, adjacent to the barb of the feather, the precise organization of the pigment granules was lacking.

In general the web barbules were most heavily pigmented near the base and only there if the amount of pigment present was small. As the amount increased, the pigmentation extended more distally, the barbicels being the last structures pigmented. In black feathers pigment seldom extended to the tips of the barbicels.

The basal portions of the fluff barbs were also usually the most heavily pigmented. Here a definite distribution pattern within the internodes was also observed. The first place to be pigmented was in the distal part of the internode. As the pigmentation increased the granules extended more proximally within the internodes. The nodes were usually the last areas to be pigmented.

A hypothesis is presented to explain the condition which some workers have called diffuse pigment. Differences in size, degree of translucence and the color of the granules would cause them to have different optical properties. Therefore, the passage of light through the granule or the reflection of light from its surface would result in a diffusion of color throughout the adjacent keratin, the amount depending upon the density of the granules, and the color upon the color of the granules.

The granule shape of different pigments seems to be color specific rather than breed specific as suggested by some workers.

Chemical Studies

The slopes of the log. E curves for feathers of 32 breeds and varieties of poultry are given.

Analysis of variance for the average slopes of the log. E curves of black, red and buff feather pigments showed these pigments to be distinctly different one from another, and is contradictory to the conclusions of some workers that these colors differ only quantitatively. Blue feather pigment is the same as that found in black feathers, and white feathers gave a significantly flatter curve than any of the color-bearing feathers.

Mixtures with known ratios of red and black feather solutions gave curves falling proportionally between those of pure black and pure red feathers. Thus a method is available for determining the relative amounts of the two pigments in unknown samples.

The steepening of the slope of the log. E curves, as affected by aging of the solutions, was demonstrated to be due largely to the change occurring in the keratin impurity since supposedly pigmentless white feathers exhibited changes of equal or greater magnitude. Such changes in colored feather or hair solutions, therefore, can not be interpreted as evidence of changes in the nature of the pigment.

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STATISTICAL ANALYSIS OF FACTORS WHICH MAKE FOR SUCCESS IN INITIAL ENCOUNTERS BETWEEN HENS[1]

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THE basic importance of initial encounters, among certain animals at least, in many different major groups of phenomena (Collias, 1943) makes it seem important to analyze the factors deciding the outcome of such encounters. To make such an analysis I have taken advantage of the remarkable fact that the first thing two strange hens will do upon meeting is to settle their future dominance relations either by fighting or by passive submission of one bird. Once established the social order is very stable and is easily ascertained at any time by noting which hens peck which without being pecked in return. The social order has been observed to persist a year or more with no change in the pecking order.

METHODS AND RESULTS OF ANALYSIS

Two hundred pair contacts were staged in a neutral pen using normal moderately inbred white leghorn hens from different flocks. These contests were conducted over a period of three years, and their original purpose was merely to serve as controls in experiments designed to test the influence of various injected hormones on the out-

come of initial pair contacts. The care and housing of the birds and the method of conducting the encounters have been described in a previous publication (Allee, Collias and Lutherman, 1939). Briefly, two hens from different flocks were caught, weighed, state of moult was noted, position in the social order in the home flock was recorded, and standard comb measurements (length plus height) were taken. The birds were then placed simultaneously in a strange pen purposely reduced to half size to insure closer association. The time of the latent period before contact and the length of the fight, if any, were recorded along with notes on general behavior of the birds toward each other. The statistical methods used in analyzing the results are described below.

By artificially increasing certain variables, it is often possible to discover which are real factors in deciding the outcome of initial encounters. This makes it possible to control those factors which can be readily controlled, and by the application of statistical analysis to evaluate the others and so to reach some idea of the relative importance of the different factors in a fairly normal situation. It is the extent to which prediction can be attained that is important, and the finer the discrimination possible, the more nearly will rules apply to the vast majority of individuals which cluster about the modes of species variability rather than merely applying to the less numerous variants at the extremes. The present report describes the progress attained toward this objective as related to success in initial encounters.

A. Nature of the Encounters

In a typical fight the birds first become oriented with respect to one another; as they examine each other the face becomes red and flushed, the neck hackles rise, the tail becomes more erect and the wings droop. The birds may then jockey for a favorable position or at once leap up and at each other and slash towards the head with the beak in an attempt to seize and bite their opponent's comb

or wattles. During the heat of battle the birds pay absolutely no attention to a human observer. The fight often lasts but a few seconds. The loser retreats and seeks to escape, its face pales, its feathers are depressed, it appears to be confused and panic stricken and looks for a place to hide, especially if closely and viciously pursued. The winner maintains much the same attitude as during the fight and only gradually does its excitement subside. Inhibition of the attack is based on experience, as well as subordination, since aggressive cocks or hens as a rule attack any strange individual, whereas attacks on flock mates are generally confined to relatively mild pecks. Fighting among hens, while often very strenuous, is much less severe than among cocks, and in the present series of encounters dominance relations were more often decided by passive submission of one bird than by an active battle.

B. Controlled Factors

1. *Sex.* As a rule cocks dominate hens when full grown (Schjelderup-Ebbe, 1935). Only hens were used in these experiments.

2. *Territorial defense.* Of 1,428 first meetings of domestic fowl observed over a period of 10 years Schjelderup-Ebbe (1922) noted that the home bird "wished" to fight in 93 per cent. while the strange bird "wished" to fight in only 32 per cent., and the home birds won 62 per cent. of the 476 battles observed. The courage of the new birds seemed lessened by their surroundings and they fought with less vigor. All the encounters reported in the present study were conducted in a neutral pen which was quite similar to a part of the home pens of the birds concerned.

3. *Social facilitation.* There is evidence that the presence of a powerful familiar despot betters the chances of its subordinates in contacts with strange individuals (Dr. A. M. Guhl, personal communication). Since in the present experiment never more than two birds were intro-

duced into the fighting pen at the same time, this factor does not complicate the results.

C. *Statistical Analysis of Uncontrolled Factors*

These factors included the following readily measured features: (1) size of comb as an indicator of male hormone output; (2) weight as a partial indicator of strength, relative size, including "impressiveness" and general physiological state; (3) social rank in the home flock as a presumed indicator of the "psychology of success"; and (4) state of moult.

Previous experience had demonstrated that very unaggressive hens could readily be caused to win all their encounters with normal hens and to rise in the social order by injecting them with testosterone propionate (Allee, Collias and Lutherman, 1939). Possibly size of comb has some "impressive" or bluff value (*ibid.*). However, Dr. A. M. Guhl (unpubl.) in this laboratory recently found that treatment of dubbed hens with testosterone caused such hens to rise in the social hierarchy; local application of this androgen induced considerable increase in comb size of normal hens, but had no influence on social rank. It had also been found that doses of thyroxin large enough to cause moulting and a marked reduction in body weight as well as to inhibit the gonads would considerably reduce the chances of a hen of winning its initial encounters (Allee, Collias and Beeman, 1940).

Size of comb was recorded in millimeters, weight in ounces, moult by arbitrary grades and social rank in terms of numbers of subordinates. For purposes of statistical analysis moult of the winner was simply treated as greater or less than the moult of the loser, while rank was adjusted for difference in size of flock by a transformed scale making use of inverse probability functions. For the latter purpose use was made of the following formula given me by Professor Wright.[2]

[2] $\mathrm{Prf}\,\dfrac{x}{\sigma}$ is the area of the unit ($\sigma = 1$) normal curve between the mean (0) and x/σ and prf⁻¹ is the symbol for the inverse function and gives the devia-

$$\text{Adjusted rank} = \text{prf}^{-1}\left(\frac{\text{Number pecked} + \frac{1}{2}}{N} - \frac{1}{2}\right)$$

We are assuming that the bird is located at a point which dichotomizes a normal distribution in such a way that the subordinates are on one side and the dominants are on the other. The bird itself is assumed to contribute equally to both sides. The proportion of subordinates is thus taken as $\dfrac{\text{number pecked} + .50}{\text{size of flock}}$. The proportion of subordinates between the mean of the hypothetical scale and the rank of the given bird is then $\dfrac{\text{number pecked} + .50}{\text{size of flock}} - \dfrac{1}{2} = \text{prf } R$. The inverse function $(\text{prf}^{-1} R)$ gives us the value of the adjusted rank on the hypothetical scale. The following diagrams may help to make this clear.
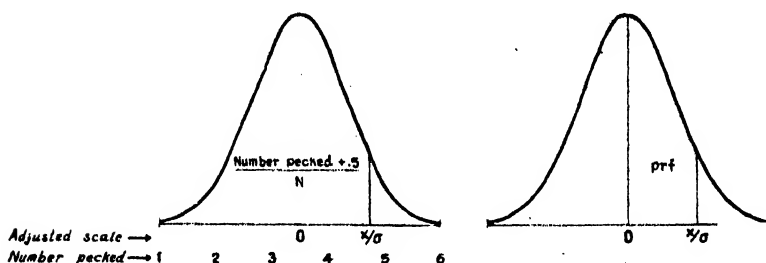
Fig. 1. Diagrams to illustrate statistical adjustment of rank in peck order to size of the home flock.

In a flock of 7 birds (maximum of 6 subordinates for highest bird) the adjusted rank for a bird which pecked 5 subordinates would be $\text{prf}^{-1}\left(\dfrac{5 + .50}{7} - .50\right) = .79$. This formula tends to bunch together the birds near the center of the social scale on the assumption that the underlying factors which make for success would exhibit a normal distribution if a large enough population were considered. All the statistical methods used in this report were based on the same assumption. All modifications of the usual

tion (in terms of σ) that underlies the designated area. Tables of these functions are provided by Davenport and Ekas (1936).

statistical methods which are used were kindly provided
to me by Professor Wright.

The first step was to correlate each factor with success.
The two hundred fights, rather than the individual birds,
have been taken as the units throughout in obtaining cor-
relation coefficients. A preliminary tabulation indicated
that even in contests conducted between the same pair of
individuals at different times the results were frequently
reversed. It must be remembered that these hens came
from different flocks and did not associate with each other
between these staged fights. If successive pair contacts
are well spaced in time, each becomes essentially an initial
contact. Hence such variability is not incompatible with
the relative permanence of social order in stable flocks of
hens. None of the data were grouped.

The variables that have been considered are the differ-
ences between the birds involved in the fight, not the
absolute measurements. Each fight may be considered
from the view-point of either bird as the one whose victory
or defeat is to be used in describing the results and from
whose grades in the various characters, those of the other
bird are to be subtracted to obtain the differences. Thus
a fight in which bird A, weighing 3 lbs. 8 oz., defeated
bird B, weighing 3 lbs., may be entered in the correlation
table relating success and difference in weight either as a
"victory" associated with a difference of + 8 oz. or a
"defeat" associated with a difference of − 8 oz. As this
is an arbitrary matter, it is legitimate to make entries
from both viewpoints, thereby making the marginal fre-
quencies of the correlation table symmetrical (as in the
case of a fraternal correlation table). In the correlations
involving success, the complete table would have 200
entries in the category "victory" and 200 entries in the
category "defeat." The total distributions of the differ-
ences in weight, comb size, rank and state of moult are
necessarily symmetrical about zero.

In determining the signs of the entries, it is assumed
that the winner of a fight stood higher than the loser on a

hypothetical scale describing the array of factors making for success. Thus "victory" is treated as positive, "defeat" as negative. A difference in rank is treated as positive if the first bird (*i.e.*, the one whose grade is to be treated as minuend) stood higher in the peck order of its flock than the second, according to the scale discussed above. With regard to the state of the plumage, it has seemed best to treat freedom from moult as highest in the scale and extreme moult as lowest. In this way, the differences which one might expect to find associated with success (*viz.*, those that go with greater weight, greater comb size, higher rank and relative freedom from moult) should exhibit positive correlations with success if, as turns out to be the case, this expectation is realized.

The correlations between success and the differences in the graded characters, weight, comb size and rank, were obtained by Pearson's coefficient of biserial correlation (in the form given by Kelley, 1923).

Let S be the difference between a first and a second bird on a hypothetical scale based on the factors making for success in fighting. A positive value implies that the first bird won, a negative value that it lost. It is assumed in the theory that the differences are distributed normally on this scale.

Let W be the weight of the first bird minus that of the second.
Let W_1 be a value of W in a case in which the first bird won.
Let W_2 be a value of W in a case in which the first bird lost.
$\overline{W}_1 = \Sigma W_1/200$, $\overline{W}_2 = \Sigma W_2/200$ are the means.
If each fight is entered in both ways $\overline{W}_1 = -\overline{W}_2$, $\overline{W} = 0$.
Thus $\sigma_W = \sqrt{\overline{\Sigma W^2/400}} = \sqrt{(\Sigma W^2_1 + \Sigma W^2_2)/400} = \sqrt{\Sigma W^2_1/200}$ is the standard deviation of all differences in weight about zero.

The formula for biserial correlation is as follows:

$$r_{SW} = \left(\frac{\overline{W}_1 - \overline{W}_2}{\sigma_W}\right)\left(\frac{pq}{z}\right)$$

where p and q are the proportions in the two categories with respect to success and z is the ordinate of the unit normal curve at the point of dichotomy. In the present case $p = q = 1/2$, $z = 1/\sqrt{2\pi} = .399$. $r_{SW} = 1.253\,\overline{W}_1/\sigma_W$.

Differences in comb size (C) and rank (R) were treated similarly.

The standard errors of these correlations were obtained from Soper's approximate formula as given by Kelley (1923).

$$SE_r = \frac{1}{\sqrt{N}}\left(\frac{\sqrt{pq}}{z} - r^2\right) = (1.253 - r^2)/\sqrt{200}.$$

The case of moult (differences represented by M) requires further consideration. Three categories were recognized: more, equal and less moult. It is assumed that these represent a trichotomy of a normal distribution of a scale of graded differences. Let a, b and c be the proportions with more, equal and less moult respectively in any distribution. Assume that the threshold between more and equal moult is at $-.50$ on this scale and that that between equal and less moult is at $+.50$. The difference between these thresholds can be expressed as follows in terms of the standard deviation (*cf.* Wright, 1934a).

$$[prf^{-1}(a + b - .50) - prf^{-1}(a - .50)]\sigma = 1$$

If a frequency between mean and threshold comes out negative as calculated from $(a + b - .50)$ or $(a - .50)$ the sign of the inverse probability function is to be taken as negative.

Among the winners there were 16% with more moult than the loser, 32% with equal moult and 52% with less moult. This yields for the standard deviation of this category (σ_{M_1}).

$$\sigma_{M_1} = 1/[-prf^{-1}(.02) + prf^{-1}(.34)] = 1.059$$

The location of the mean of the winners on our hypothetical scale can now be obtained.

$$\overline{M}_1 = .50 - \sigma_{M_1}prf^{-1}[a + b - .50] = .50 + 1.059\ prf^{-1}(.02) = .553$$

Because of symmetry, the mean of the losers $(\overline{M}_2)$ is $-.553$ on this scale.

The variance of the total (σ_M^2) is compounded of the average within the rows (both $1.059^2 = 1.122$) and that between them (which is $.553^2 = .306$). Thus $\sigma_M^2 = 1.122 + .306 = 1.428$, $\sigma_M = 1.195$.

The standard deviation of the total could also be calculated directly from the symmetrical trichotomy of the total $(a = .34, b = .32, c = .34)$.

$$\sigma_M = 1/[prf^{-1}\ .16 + prf^{-1}\ .16] = 1.212$$

The discrepancy between these estimates is due to the fact that if the distributions of the separate rows are normal (as assumed in calculating their means) the distribution of the total is more or less platykurtic, instead of normal (as assumed in the second estimate of σ_M). Properly it is this total (based on birds taken in both orders with respect to success and therefore equivalent to ones taken in random order) that should be considered as normal, but for consistency with the calculation of the row means, the estimate $\sigma_M = 1.195$ is used here. The error in assuming normality of the distributions within the separate rows does not appear to be important as indicated in some measure by the comparison of the two estimates of the total standard deviation.

The biserial correlation may now be estimated.

$$r_{BM} = 1.253 \ M_1/\sigma_M = .580 \pm .065$$

This has been assigned a standard error by the same formula as in the preceding cases, although this is undoubtedly somewhat of an underestimate because of the nature of the scale. There is, however, no doubt of the significance of this correlation.[3]

In order to estimate the relative importance of the different factors it was necessary to take into account their correlations with each other. These correlations were obtained by use of the ordinary product moment formula in the case of differences in weight, comb, and social rank.
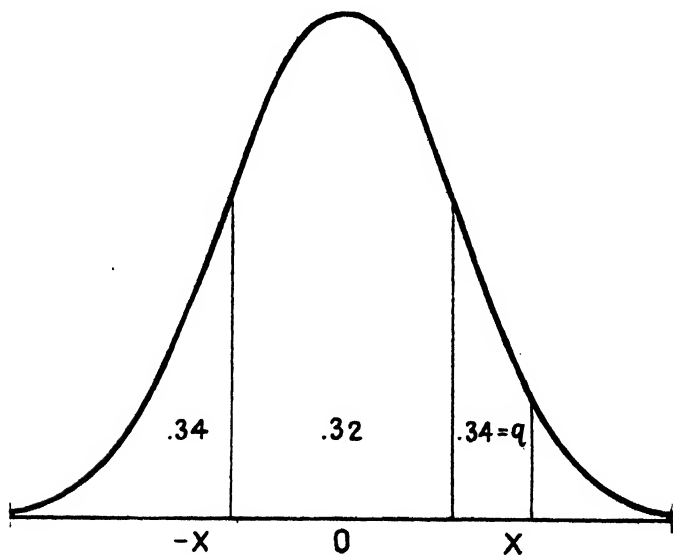
Fig. 2. Transformation of values for moult to a numerical scale on the normal probability curve.

Pearson's broad category method was used in the cases in which moult is involved. The marginal frequencies (34% more, 32% equal and 34% less moult) are here assumed to trichotomize a normal probability curve with unit standard deviation, a different scale from that used above. The mean of the middle category is obviously zero. The mean deviation (d) for the category "less moult" is given by the following formula in which q is

[3] Professor Wright, personal communication.

the tail frequency (.34) and z is the ordinate at the threshold.

$$d = z/q = .3664/.34 = 1.078$$

The deviation of the category "more moult" is obviously -1.078.

$$\sigma_d^2 = (.34d^2 + 0 + .34d^2) = .790$$

is the variance as estimated from the mean deviations. The true variance is 1.

According to Pearson's formula,

$$r_{MW} = \frac{\Sigma dW}{N\sigma_W \sigma_d^2}.$$

The formulae for r_{MC} and r_{MR} are similar.

All these correlations between factors have been assigned standard errors by the usual formula, $\sigma_r = (1 - r^2)/\sqrt{N}$ although this is undoubtedly slightly too low in the cases involving moult, because of the coarseness of the scale.

We assume that the array of factors upon which the result of a fight depends includes cases in which differences in weight, comb size, state of moult and social rank are close indicators. In Figure 3 the correlations are known and are indicated by curved lines. The object is to find the value of coefficients measuring the influence along the paths indicated by arrows. An estimate can be obtained by the method of path coefficients (Wright, 1921, 1934a, 1934b) which in a symmetrical system such as used here is essentially the same as that of multiple regression, except for qualifications from the hypothetical character of the scale in the case of the differences in state of moult, social rank and success. The correlation between success and one of the factors such as weight may be analyzed into a direct contribution measured by the coefficient for the path from weight to success and three indirect contributions measured by the products of the coefficients along the paths from W through C, M, and R respectively to S. The correlations between success and the four factors provide four simultaneous equations which are identical with the normal equations of multiple regression.

$$r_{SW} = p_{SW} + p_{SC}r_{WC} + p_{SM}r_{WM} + p_{SR}r_{WR}$$
$$r_{SC} = p_{SW}r_{WC} + p_{SC} + p_{SM}r_{CM} + p_{SR}r_{CR}$$
$$r_{SM} = p_{SW}r_{WM} + p_{SC}r_{CM} + p_{SM} + p_{SR}r_{MR}$$
$$r_{SR} = p_{SW}r_{WR} + p_{SC}r_{CR} + p_{SM}r_{MR} + p_{SR}$$

Table 1 summarizes the correlation coefficients and the path coefficients resulting from solutions of the normal equations.

TABLE 1

CORRELATIONS AND PATH COEFFICIENTS RELATING DIFFERENCES IN CHARACTERS TO SUCCESS IN FIGHTS FOLLOWING INITIAL ENCOUNTERS

Difference	Correlation with success	Path coefficient relating to success
slightness of moult	.580 ± .065	.417
comb size	.593 ± .064	.354
weight	.474 ± .073	.111
rank in own flock	.262 ± .084	.209
Multiple correlation = $\sqrt{\Sigma r p}$ = .748		

TABLE 2

CROSS CORRELATIONS AMONG THE CHARACTER DIFFERENCES

Character differences	
Slightness of moult and comb	.379 ± .061
Slightness of moult and weight	.387 ± .060
Slightness of moult and rank	−.064 ± .070
Comb and weight	.440 ± .057
Comb and rank	.156 ± .069
Weight and rank	.220 ± .067

TABLE 3

DEGREE OF DETERMINATION OF SUCCESS BY INDICATED FACTORS

Moult	.174
Comb	.125
Weight	.012
Social rank	.044
Moult and comb, joint residual	.112
Moult and weight, joint residual	.036
Moult and rank, joint residual	−.011
Comb and weight, joint residual	.035
Comb and rank, joint residual	.023
Weight and rank, joint residual	.010
Total determination (= $.748^2$ =)	.560
Residual determination =	.440
	1.000

These results need some interpretation. The correlation coefficients measure the total influence direct and indirect, of the various factors. The multiple correlation coefficient (.748) indicates the correlation between the best linear function of all of the factors and success.

Because of inter-correlations, the correlation coefficients do not however give a reliable evaluation of direct effects of the factors. The relatively high correlations between moult, weight, and comb size may be an expres-
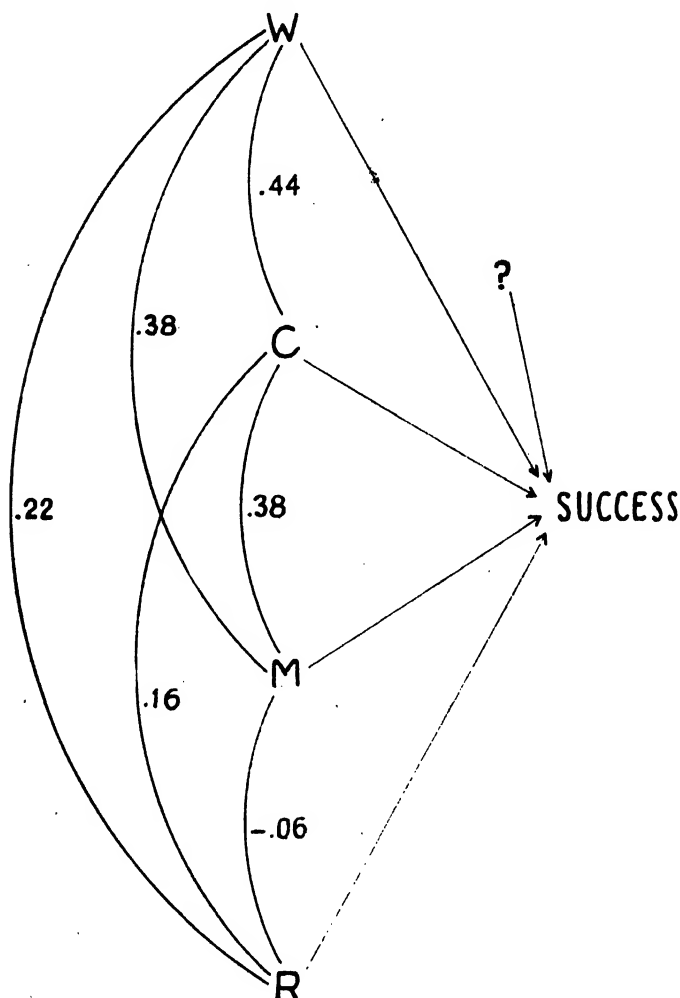
Fig. 3.   Interrelations of factors influencing result of initial encounters.

sion of pituitary action but they can be more directly explained as being due in large part to a common factor which is presumably the amount of thyroxin which is present since this hormone in fairly large doses will in-

duce moulting, reduce weight and suppress the gonads, all of which are more or less associated with loss of combat. The correlation between social rank and weight (.220) between social rank and comb size (.156) are low. The correlation that does exist may be a result of the fact that differences in weight and androgen which helped decide the original rank have persisted in part. Social rank once decided is much less variable than is weight, size of comb, or state of moult, hence the correlation is low. Then, too, birds high in the peck order have precedence to food and probably thrive better. The very low correlation between moult and social rank (− .064) is probably of no significance.

Inspection of the tables indicate that according to the path coefficients, moult (absence) was the most important factor deciding success, followed by male hormone indicated by comb, social rank and weight in that order. The small influence of weight is noteworthy. Moult exerts the greatest relative influence, but exactly how it does this is not known since it has been statistically freed from its actions through weight or the gonads. Perhaps it tends to make the bird more retiring since moult is really a kind of physiological illness, perhaps it makes the bird more sensitive to blows received, and perhaps the loss of feathers renders a bird less able to maintain equilibrium and to fight effectively. Size of comb is one of the best convenient indicators of male hormone output which we know at present. Hens secrete male as well as female hormone and injection of androgen has been shown to have a very marked and stimulating effect on the outcome of initial encounters unlike injection of estrogen (Allee, Collias and Lutherman, 1939; Allee and Collias, 1940). Social rank is perhaps a somewhat objectionable criterion since it may conceal factors of the very nature we are trying to uncover. However, it was the most convenient indicator of the ''psychology of success'' available, and furthermore this objection is obviated to a substantial degree by the low correlations of moult, comb and weight

with social rank as compared to their relatively high correlations with success in initial encounters. The rather small importance of social rank as an independent factor as indicated by its path coefficient is also noteworthy in this respect.

The degrees of determination of the variance by the various factors reduce the results to a percentage basis and give a good idea of how the different factors influence variability in degree of success, acting singly and in combination. Acting singly, they necessarily exhibit the same order as their path coefficients since they are merely the squares of the former. Joint determination represents the contribution to variability (plus or minus) due to correlated occurrence of the two factors under consideration. The order of joint determinations obviously may differ from the order of the intercorrelations since the latter do not measure degree of influence on success. For example, moult and weight are more closely correlated than moult and comb, yet the joint determination by the latter pair is almost four times as great as that by the former pair which involves weight, a relatively ineffectual factor. One of the important properties of the degree of determination is that the sum of the components must be equal to 1. The general equation is $\Sigma d_{x.A} + d_{x.\overline{AB}} = 1$, which is read, the sum of the determination of x due to A and the sum of the determination of x due to the correlation between A and B must be equal to 1 (Wright, 1921). This gives a means of estimating the percentages of unknown factors (residual) by subtraction. In the present case 56.0 per cent. of the factors are accounted for by the single or correlated action of moult, comb size, weight and social rank, and presumably the underlying factors of which they serve as more or less imperfect indicators are responsible for a somewhat similar percentage. It follows that 44 per cent. of the factors were not measured in this analysis or were not known.

D. Unmeasured and Unknown Factors

One possible way to account for the unknown factors lies in the probability that more accurate indicators of the underlying factors would have given higher values for the paths, cutting down the amount assigned to residual factors. This is not to assume that still other factors do not exist which are unrelated to these indicators.

There are certain known but unmeasured factors which influence success and may have complicated the results to an unknown extent in the present case. Among these are age, the behavior of the other bird, and previous fighting experience.

Young pullets up to at least 9 months of age are somewhat refractory to injected androgen in terms of the stimulation of aggressive behavior (Allee, Collias, and Lutherman, 1939). Some of the birds used were barely older than this and included flock-mates of the treated birds. When encounters involving birds less than one year of age with older birds were omitted, the multiple correlation coefficient fell slightly from 0.75 to 0.70. The path coefficients were almost identical except that that for the comb size fell to 0.31 from 0.35. Possibly difference in general size weakened the accuracy of the comb as an indicator since the older hens tended to be somewhat heavier and larger. On the other hand, senility may play a role. Degenerative changes occur in the pituitary of old fowl (Payne, 1941). There is some recent evidence which suggests the possibility that senile animals may become less sensitive to injected androgens (Hoskins, Levine and Bevin, 1939). One flock of our hens was approaching senility, but were still laying, during the last set of encounters in which they were used. However, no effect of age differences on success is evident from the gross data; of the 200 encounters, 36.5 per cent. were won by older birds, 39.5 per cent. by younger birds, and in 25 per cent, of the cases the opponents were of about the same age.

The behavior of the other bird is undoubtedly important. A hesitant individual will often take heart and attack when it observes that its opponent seems frightened. In only 33.5 per cent. of the encounters did an actual battle ensue, and 78 per cent. of the encounters were won by the bird that started the encounter. The longer the latent period before contact began the shorter a fight was likely to be and the greater the probability that one bird would submit passively.

Experience in winning or losing can be an important factor at times, and by purposeful application of this factor the results of a later encounter between a given pair of birds can often be reversed in a later encounter. It is a little difficult to extract suitable test cases in encounters of hens. Seven cases were tested in which the original loser had won from two to five encounters while the original winner had lost from three to six encounters just before the second meeting, and the results were reversed in every case. Ginsburg and Allee (1942) have recently brought out the marked importance of this factor of conditioning in mice by a series of carefully controlled experiments. Whether conditioning played much of a role in the present series of encounters is somewhat debatable, but a conscious effort was made to control this factor by not fighting any bird more than once a day. The more immediate history of a bird might perhaps be expected to exert the greatest influence; however, it is interesting to note in this connection that the correlation between winning a given encounter and success in the preceding encounter was exactly the same as the correlation with the second preceding encounter, that is, 0.66. This tetrachoric correlation was conveniently secured by use of computing diagrams prepared by Thurstone and Chesire.

Endurance is probably a factor when an actual fight takes place. By fighting the same tough hen over and over again in succession Schjelderup-Ebbe (1922) was able to make her yield and be subordinate to weaker

birds. During a battle it is obvious from the increasing slowness of movements of the birds that they are becoming more and more tired. Since none of the hens were fought more than once a day in the present series of encounters some measure of control over this factor was afforded.

The nature and importance of more completely unknown factors can merely be speculated on at present—differences in fighting skill, chance blows, differences in sensitivity to hormones, wildness, mild indisposition akin to illness, resemblance of the opponent to a despot or subordinate in the home flock, the details of past history, minor external disturbances, slight differences in handling, and errors in measurement perhaps have an aggregate effect of significant dimensions.

APPLICATIONS OF THE METHOD AND RESULTS

The significance and general implications of the problem will be developed elsewhere (Collias, 1943). The general importance of the statistical method employed is that it provides something of a model whereby the relative importance of the various factors which decide social dominance relations in various situations of ecological significance may be quantitatively evaluated. The present statistical method of evaluating the factors which make for high social level would have to be modified for each particular case, but this would probably not entail insurmountable difficulties.

One of the biggest complications and not adequately dealt with in this report since its influence was experimentally eliminated, is the interrelationship of territory. Schjeldrup-Ebbe's data indicate the possibility that in chickens, which have not generally been regarded as territorial birds, this factor may often outweigh all the others. However, even in nature, birds may on rare occasions be displaced from their territories by stronger rivals. Perhaps territorial influence could be measured by the distance from the territorial center.

More specific parallels of the general results to natural situations, at least cases where one factor apparently dominates the situation, can be cited even in the present inadequate state of investigation of social hierarchies in the vertebrates. At first thought, the size and weight factor is perhaps most obvious, *e.g.*, a general order of precedence to food in rough order of size differences is very common, for example, in birds of different species at a feeding shelf (Nice, 1929), or in different species of ducks and geese on a park lagoon (Jenkins, in press). The factor of male hormone is probably the important one in many situations. Males usually dominate females, and Lorenz (1931) states that young jackdaws rise in the peck order as they become reproductively activated, and much the same thing has been found by Shoemaker for canaries (1939). Coveys of quail and winter flocks of many other birds break up with the onset of the breeding season and the parallel rise in gonad activity and individual aggressiveness. Fighting is very common in groups of young male mammals (Alverdes, 1935). Examples of the action of thyroxin are more obscure and lie on more unstable assumptions, because of the complexity of its effects. In general, birds when moulting tend to retire to dense cover and hence to avoid conflict with other members of their species. The same retiring habit is seen during incubation when gonad activity is likewise reduced. Schjelderup-Ebbe (1922, 1935) has described a decided increase in aggressiveness of broody hens and such accords with the popular idea, but some evidence has been gathered in this laboratory which, while inadequate in itself, suggests the need for caution in relating such things as the defense of her chicks by a broody hen to position in the social hierarchy of the flock. The effective stimuli for types of aggressive behavior with different physiological bases may be very different.

SUMMARY

Initial encounters lie at the basis of the social order in flocks of chickens, as is known to be the case with a num-

ber of other vertebrates. To gain some insight into the factors which decide the outcome of initial encounters 200 pair contacts were staged in a neutral pen using normal, moderately inbred white leghorn hens from different flocks. Controlled factors included sex, territorial familiarity, and social facilitation. Evaluation of the less easily controlled factors was made mainly by the use of a modified biserial correlation formula. The degrees to which success in encounters was controlled by the more important factors were determined by the method of path coefficients which in the present instance was the same as the method of multiple regression.

Factors of major importance were male hormone output as indicated by comb size and thyroxin secretion as indicated by the complex of changes which accompany moulting. Social rank in the home flock had much less influence, and weight was of only small importance.

The multiple correlation coefficient of success with the four factors analyzed was 0.75. Forty-four per cent. of all factors were unknown or unmeasured in this analysis.

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PROOF FOR MULTIPLE ALLELISM OF SEX-DIFFERENTIATING FACTORS IN HABROBRACON[1]

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In the wasp Habrobracon, sex determination is complementary. Haploid males, similar in phenotype, are of different recessive genotypes. Diploid males, likewise similar in phenotype, are of different homozygous recessive genotypes, corresponding to those of the haploid males. Females are produced by any of the heterozygous combinations of the different male sex genotypes.

Snell (1935) proposed a multiple factor theory for sex determination in Habrobracon, according to which females are developed if the zygote is heterozygous for one or more independently segregating factor pairs, $xa/xb, ya/yb, za/zb$. Males must be homozygous (diploid) or azygous (haploid).

Whiting (1940) analyzed previously published data, showing them to be consistent with a multiple allele theory. According to this theory, the normal haploid males would have any one of a series of sex alleles (xa, xb, xc, xd, etc.), the corresponding diploid males would be homozygous (xa/xa, xb/xb, xc/xc, etc.), and the females heterozygous (xa/xb, xa/xc, xb/xc, etc.). With n alleles there would be n different kinds of haploid males, n corresponding homozygous diploid males and $\dfrac{n^2 - n}{2}$ heterozygous "double dominant" female-producing combinations.

No intersexes have ever been found associated with any of the sex differentiating genotypes of Habrobracon. The diploid males are in every way similar to the

haploid males, except for differences usually associated
with doubled chromosome number (cell size, etc.). They
show no evidence of feminization either in primary or in
secondary sex traits.

Fraternities containing diploid males have previously
been called closecross fraternities because in general the
parents have been closely related, while fraternities con-
taining no diploid males have been called outcross fra-
ternities since these are usually produced from unrelated
or distantly related parents. On the basis of the multiple
allele theory, these two types of fraternities may now be
more exactly designated two-allele and three-allele fra-
ternities.

Sex ratio in Habrobracon depends, among other things,
upon ratio of eggs fertilized. Under standard incubator
conditions this is roughly two thirds, but there is much
fluctuation. When conditions are otherwise uniform,
ratio of females to haploid males in three-allele fraterni-
ties is twice that in two-allele fraternities since all zygotes
are heterozygous (female-producing) in the former case,
but only half are heterozygous in the latter. The homo-
zygous (diploid male-producing) combinations constitut-
ing the remaining half of the zygotes in the two-allele
fraternities are of low viability, so that there are many
unhatchable eggs and ratio of diploid males to females is
very much lowered from unity. This "diploid male
viability ratio" measures the viability of the diploid
males which is very variable relative to that of the females
which is high and very stable.

PRELIMINARY EXPERIMENT

Every F_2 male (haploid) from crosses of stocks with
different sex alleles, *xa/xb* and *xc/xd,* when mated to
females of both parental stocks, should fall into one or
the other of two classes, those (*xc* and *xd* males) siring
diploid sons with *xc/xd* females but not with *xa/xb*, and
those (*xa* and *xb* males) siring diploid sons with *xa/xb*
females but not with *xc/xd*.

No diploid males have ever been obtained from crosses between stocks 17-ivory and 36-veinless, indicating that these stocks have different sex alleles and they were therefore selected for use in the following experiment. The mutant genes ivory, o^i, and veinless, vl, served as markers to separate the haploid males from their diploid brothers.

Each of 17 F_2 males from reciprocal crosses when backcrossed sired diploid sons by stock 36-veinless females (+ ♀♀ 1390, vl ♂♂ 902, + ♂♂ 95) but failed to sire them by stock 17-ivory females (+ ♀♀ 1644, o^i ♂♂ 912). Ratio of females to haploid males in the latter cross, 1.803, is higher than that in the former, 1.541, indicating replacement of diploid males by females in the two-allele fraternities. In this case the low viability of the veinless males decreases the expected 2:1 proportion between the female ratios of the three-allele and two-allele fraternities to 1.170:1.

Each of 17 F_2 males when back-crossed sired diploid sons by stock 17-ivory females (+ ♀♀ 1102, o^i ♂♂ 1213, + ♂♂ 105) but failed to sire them by stock 36-veinless females (+ ♀♀ 2193, vl ♂♂ 796). Low viability of veinless exaggerates the difference in female to haploid male ratio in these crosses 3.03:1 instead of 2:1.

Two males sired no diploid sons by females of either parental stock. Among their offspring from veinless females the daughters were 3.27 times as numerous as the sons (+ ♀♀ 108, vl ♂♂ 33) and from ivory females the daughters were only 0.91 times as numerous (+ ♀♀ 95, o^i ♂♂ 104). Difference in ratio of daughters to haploid sons indicates that these two males had the same sex alleles as the ivory females and that the lack of diploid sons is an error of sampling.

Ratio of diploid males to females (diploid male viability ratio) is 0.090 for all the two-allele fraternities.

According to Snell's (1935) multiple factor hypothesis crosses of two stocks with the same sex-differentiating pair will produce only female-developing zygotes if the

stocks differ by one or more sex factors for which each
is homozygous. With two differences involved, the stocks
may be designated *xa/xb ya/ya za/za* and *xa/xb yb/yb
zb/zb*. All zygotes from crosses of these two stocks will
be female because *ya/yb za/zb* although half would be
homozygous for *x*. If the *x* pair be disregarded because
both members are present in each stock, the F_2 males will
fall into four equal classes, *ya.za* and *yb.zb* which will sire
diploid sons with one stock but not with the other and
ya.zb and *yb.za* which will sire diploid sons with neither
stock. Thus only half of the F_2 males can sire diploid
sons in the tests. If the stocks differ by three factors
only one fourth can sire diploid sons, if by four factors,
only one eighth, etc. The data presented above preclude
the possibility that two or more different pairs of sex
factors characterize the stocks, if the sex-differentiating
factors are the same.

If the sex-differentiating factors of the two stocks are
different, *xa/xb ya/ya za/za* and *xa/xa ya/yb zb/zb*, cer-
tain F_2 males produced by some of the F_1 females can
sire diploid sons by females of neither stock. Data
presented above are not regarded as adequate entirely to
preclude this possibility. These data are also consistent
with the two factor hypothesis of *xa/xb ya/ya* and *xa/xb
yb/yb* stocks according to which half of the F_2 males
will sire diploid sons by *xa/xb ya/ya* females but not by
xa/xb yb/yb females and half will show the reverse result.
This is also as expected for quadruple alleles.

USE OF THE RECESSIVE SEX-LINKED GENE FUSED IN TESTING MULTIPLE ALLELISM OF THE SEX FACTORS

In two-allele fraternities sex-linkage of fused is obvious
with about 10 per cent. crossing-over. If fused is asso-
ciated with the same sex allele in the female as in the
male, the majority of the daughters will be wild type, of
the diploid sons fused.

$$P_1 \qquad \frac{x}{xb\,fu} \; ♀ \times xb\,fu \; ♂$$

	females	diploid males
F_1		
non-crossovers	$9 \; \dfrac{xa+}{xb\,fu}$	$9 \; \dfrac{xb\,fu}{xb\,fu}$
crossovers	$1 \; \dfrac{xa\,fu}{xb\,fu}$	$1 \; \dfrac{xb+}{xb\,fu}$

If fused is associated with different sex allele in the parents, the majority of the daughters will be fused, of the diploid sons wild type.

$$P_1 \qquad \frac{xa+}{xb\,fu} \; ♀ \times xa\,fu \; ♂$$

	females	diploid males
F_1.		
non-crossovers	$9 \; \dfrac{xb\,fu}{xa\,fu}$	$9 \; \dfrac{xa+}{xa\,fu}$
crossovers	$1 \; \dfrac{xb+}{xa\,fu}$	$1 \; \dfrac{xa\,fu}{xa\,fu}$

In three-allele fraternities sex-linkage is masked since all zygous offspring are female.

$$P_1 \qquad \frac{xa+}{xb\,fu} \; ♀ \times xc\,fu \; ♂$$

	females	females
F_1		
non-crossovers	$9 \; \dfrac{xa+}{xc\,fu}$	$9 \; \dfrac{xb\,fu}{xc\,fu}$
crossovers	$1 \; \dfrac{xa\,fu}{xc\,fu}$	$1 \; \dfrac{xb+}{xc\,fu}$

Bostian (1939) by a prolonged inbreeding experiment discovered the presence in his stock of triple alleles governing sex. Females heterozygous for fused were in each generation crossed with fused males of the same stock. His line was continued from those fraternities in which all diploid offspring were females, non-fused and fused in equal numbers. Triple alleles, *xa*, *xb*, *xc*, were maintained by his system of mating according to the following scheme:

$$\text{Odd generations} \quad \frac{xa+}{xb\,fu} \; ♀ \times xc\,fu \; ♂$$

$$\text{Even generations} \quad \frac{xa+}{xc\,fu} \; ♀ \times xb\,fu \; ♂$$

According to these formulae all zygous offspring would be heterozygous and hence female but in every generation a few two-allele fraternities appeared showing diploid males always associated with sex-linkage of fused.

If sex-differentiation were shifted to a second pair of factors $\left(\dfrac{xa + ya}{xa\,fu}\dfrac{}{yb}\,♀ \times xa\,fu\,ya\,♂\right)$, diploid males should appear without sex-linkage of fused. Failure to obtain this condition despite prolonged selection proves that only one allelic series was involved in Bostian's experiment.

Whiting (1940) showed quadruple alleles in two stocks. Fused was similarly sex-linked in each stock, indicating that a single series was involved. Matings of heterozygous females with fused males within each stock resulted in two-allele fraternities with zygous males as well as females in proportions indicating linkage: $♀♀ + 9$, $fu\,1, ♂♂ + 1, fu\,9$ or $♀♀ + 1, fu\,9, ♂♂ + 9, fu\,1$. In crosses between the stocks the linkage was masked since all zygous offspring were female, non-fused and fused in equal numbers, as is characteristic of three-allele fraternities.

Dr. Whiting now has nine sex alleles in five stocks. Linkage with fused is shown in closecrosses (two-allele) but is masked in outcrosses (three-allele) except that stocks xa/xb and xa/xi have one sex allele in common. Diploid males, xa/xa, appear in half the crosses between these two stocks.

Dr. Whiting's data, showing that the sex-differentiating pairs of his five stocks are fused-linked, indicates that a single pair or series is involved in sex-differentiation within each stock. His stocks might conceivably be identical in x, all xa/xb, but differ in other factors. This is extremely unlikely, however, in view of the high probability of obtaining homozygosis of x with consequent non-sex-linkage of fused, as would be expected if y, for example, were the sex-differentiator, $\dfrac{xa + ya}{xa\,fu}\dfrac{}{yb}$.

BACKCROSS EXPERIMENT INVOLVING FUSED

Two stocks were supplied by Dr. Whiting, marked by different non-allelic recessive eye colors and each having the sex-linked female-sterile gene fused, which was maintained by crossing heterozygous females, $+/fu$, to fused males, fu. For the experiment orange-eyed xa/xb females heterozygous for fused, $\dfrac{o\ xa\ +}{o\ xb\ fu}$, of one stock were crossed with red-eyed xe or xf fused males of the other stock and the reciprocal was also made. The F_1 black-eyed females and the haploid males, orange or red following their maternal eye color, are fused and non-fused in approximately equal numbers. No diploid (black-eyed) males occur in F_1.

Ninety-seven black-eyed fused F_2 males from the reciprocal crosses produced a sufficient number of diploids with both P_1 stock females to be included in the summary of data (Table 1). Forty-eight of these males were from the cross orange females by red fused males, and forty-nine from the reciprocal cross. Since in the F_1 females heterozygous for fused, the fused came from the P_1 sire, and since the black-eyed fused F_2 males were used for backcrossing, the expectation was that, except for crossovers, these fused males would carry the sex allele from the paternal P_1 stock. This occurred in 86 of the 97 cases. The remaining 11 (crossovers) had a maternal P_1 sex allele. This, a crossover value of 11.3 per cent., is in good agreement with the expectation based on previous crossover values for fused and the sex factor.

Because of the necessity of maintaining the female-sterile gene fused by mating non-fused females to fused males, it is to be expected that the majority of heterozygous stock females will have this factor associated with the same sex allele as that of the 86 non-crossover F_2 fused males. The two-allele fraternities from these males should then show deficiency of fused daughters and corresponding excess of fused sons. Such proved to be the case for all the matings of 67 males and for some

TABLE 1

DATA FOR BACKCROSSES OF BLACK-EYED FUSED F₂ MALES FROM RECIPROCAL CROSSES OF ORANGE XA/XB AND RED XE/XF STOCKS

F_2 males	Three-allele fraternities					Two-allele fraternities						
	Females		Haploid males		females / haploid males	Females		Diploid males		Haploid males		females / haploid males
	non-fu	fu	non-fu	fu		non-fu	fu	non-fu	fu	non-fu	fu	
with paternal sex allele.												
*67	3829	3099	1670	1480	2.53	3830	383	35	404	2000	1690	1.28
†15	706	641	326	279	2.35	114	740	42	12	373	311	1.47
‡4	204	166	77	49	3.01	18	86	9	2	45	35	1.28
						107	20	2	9	54	69	
with maternal sex allele.												
*2	107	68	32	39	2.44	36	8	0	5	12	19	1.65
†8	368	293	141	141	2.86	60	322	16	7	190	165	1.01
‡1	46	45	44	37	1.12	26	18	0	0	3	10	1.21
							7		4	22	12	

* Males siring deficiency of fused daughters in two-allele fraternities.
† Males siring excess of fused daughters in two-allele fraternities.
‡ Males siring deficiency and excess of fused daughters in different two-allele fraternities. The two types of two-allele fraternities are recorded separately.

of the matings of four others. The remaining tests of the non-crossover F_2 males showed that crossingover had occurred in the paternal stock so that a minority of females produced fraternities with the reverse type of linkage, excess of fused daughters, deficiency of fused diploid sons.

Since fused in the F_1 females becomes assoicated by crossingover with the sex allele from the maternal stock usually combined with non-fused, the expectation is that excess of fused daughters and of wild-type diploid sons will be sired by the crossover F_2 fused males. Eight of the eleven crossover males gave this result with all their mates and one with one of his two mates.

Ratio of females to haploid males in the fraternities showing linkage is 1.294 ± 0.055 and it is approximately twice as high, 2.537 ± 0.092, in the fraternities with linkage masked. This is to be expected, since all the fertilized eggs are female-producing in the latter case, but only half in the former.

The mean ratio of diploid males to their sisters is 0.104 ± 0.007 for the fraternities showing linkage.

The crossover value for fused and x based on the fraternities showing linkage is 10.6 per cent. (674 crossovers, 5,714 non-crossovers).

If the sex factor pair xa/xb linked with fused were the sex differentiator in both stocks and one other pair of sex factors were present, homozygous for different alleles in the two stocks, ya/ya and yb/yb, fraternities with diploid males and showing linkage should be sired by 50 per cent. of the F_2 fused males by paternal stock females and 50 per cent. by maternal stock females. $\left(P_1 \text{ stocks} \right.$ $\dfrac{xa + ya}{xb\,fu\,\,ya}$ and $\left. \dfrac{xa + yb}{xb\,fu\,\,yb} \right)$. Actually the percentages were 88.7 and 11.3.

If the sex factor pair linked with fused were not the sex differentiator (this is, however, contrary to Dr. Whiting's evidence), the non-crossover F_2 fused males

would sire diploid sons when backcrossed to paternal stock females, but not to maternal stock, while the cross-over F_2 fused males would sire diploid sons when back-crossed to maternal stock females, but not to paternal stock. But in this case, since the sex differentiator in the stock was not linked to fused, linkage would be masked whether diploid males were produced or not. $\left(P_1 \text{ stocks } \dfrac{xa + ya}{xa\,fu\,yb} \text{ and } \dfrac{xb + ya}{xb\,fu\,yb}\right)$. The data show sex-linkage of fused in all the fraternities with diploid males.

The results obtained in these experiments cannot be interpreted on a basis of more than a single series of allelic factors.

THEORETICAL CONSIDERATIONS

It seems likely that sex is rarely, if ever, differentiated by a single gene. Such may indeed be the first evolutionary step initiating the process, but soon other differences will be accumulated until a differential chromosome segment is developed with many genes linked together into a factor segregating as a unit in meiosis. Thus in *Drosophila melanogaster*, the entire X-chromosome containing numerous but unnumbered sex genes segregates from the Y in spermatogenesis. Crossing-over between the two X's in the female tends to equalize differences, producing uniformity in the population and preventing the evolution of diverse X-chromosome races.

In Lymantria polygenic sex differences have been accumulated distinguishing geographically separated races in each one of which sex balance is normal. There is here a series of homologous Z factors balanced against homologous W factors, but they can not, for the most part, function as alleles in crosses because of intersexuality and consequent sterility of the hybrids. It is possible also that crossingover may occur to some extent in the hybrid males between the Z's of different racial origin so that they do not function as unified alleles.

In species with sex highly differentiated morphologically and even in relatively "undifferentiated" races

there tends to be a dominating unit factor difference, the retention and evolution of which serves to check the production of intersexual and sterile types. The Hymenoptera are a highly evolved group of insects showing wide sex differentiation, both structural and functional. A multiple factor scheme therefore seems unlikely *a priori*.

Since the differential maturation hypothesis (to account for the replacement of diploid males by females) has now been disproved, the only alternative to multiple factors would seem to be a series of multiple alleles, duplicating each other in phenotypic effect but consisting basically of an unknown number of diverse genes. These must have been so selected as to have a complementary effect in female production, this complementary effect being maintained by haploid parthenogensis subjecting the sex differentiators (Whiting, 1935) "equally to natural selection. They can not, therefore, degenerate by the accumulation of recessive lethals. Were it not for parthenogenesis, the complementary scheme might shift over to one of simple dominance, the crossing of a heterozygote with a recessive as in other forms."

SUMMARY

Evidence is presented showing that sex-differentiating factors in Habrobracon are allelic to each other in a single series (multiple alleles) rather than occurring in separately segregating pairs (multiple factors). Backcross breeding tests of the composition of F_2 males proves that quadruple alleles are involved in the crosses reported in this paper.

Bostian's proof of triple alleles in his inbreeding experiment and Whiting's evidence for nine alleles linked with fused are discussed.

In contrast to a multiple factor hypothesis, the theory of multiple alleles is regarded as more consistent with general principles of the evolution of sex differentiation in the entire group of Hymenoptera.

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REVIEWS AND COMMENTS

EDITED BY CARL L. HUBBS

IN this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution.

REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

Criteria for Vertebrate Subspecies, Species, and Genera. By CHARLES M. BOGERT, W. FRANK BLAIR, EMMETT REID DUNN, E. RAYMOND HALL, CARL L. HUBBS, ERNST MAYR, and GEORGE GAYLORD SIMPSON. Ann. New York Acad. Sci., 44, 1943: 105–188. $1.00.

THE seven papers comprising this symposium bring to notice taxonomic practices and viewpoints employed by workers concerned with each major group of vertebrate animals. Consecutive reading of the items is likely to create a kaleidoscopic effect, for of necessity each contributor in preparing his paper was largely unapprised of the manner of treatment adopted by his associates. But even though organization and emphasis vary greatly among the authors, one is encouraged to find acceptance of the same fundamental concepts of speciation. None seems to question a normal, or at least frequent, continuity in evolution from subspecies to species to genus, nor do any fail to appreciate, at least in a general way, the genetic mechanisms involved in speciation and the necessity for statistical understanding—aspects especially developed by Blair and Simpson, respectively. Divergence comes in shaping definitions, in drawing lines between stages in the evolutionary continuum, and in rating the several criteria that may be utilized. These differences depend largely on whether the worker emphasizes the practical or the theoretical in taxonomy and whether he is willing to declare, rather subjectively, that two geographically isolated forms would interbreed if

thrown together or, contrarily, feels constrained to adhere closely to ascertained facts concerning their morphological divergence or overlap.

In sifting the discussions for points of agreement, we find that the genus is accorded little attention and ordinarily is dismissed as a category of convenience without real boundaries in the system and dependent on uncircumscribable similarities and differences in morphology (Dunn, Hall, Hubbs, Mayr). Simpson's theoretical definition of the genus as a category within which there are genetically discontinuous units does not account for monotypic genera, a few of which at least would seem legitimate. Authors who touch on the question of the size of genera favor large units because they are more useful in showing relationships.

It is agreed that the morphological characters of subspecies, species and genera are not intrinsically different. It is the continuity or discontinuity, genetically and morphologically, of the assemblages of individuals bearing these characters which figures in the definitions.

No one declares in favor of quadrinomials for minor differentiations below the subspecies. As a rule of thumb, differentiation must have proceeded to a point where about 75 per cent. of the animals in a population are morphologically distinguishable from those in adjacent populations before subspecific designations are given (Hubbs, Dunn, Mayr). Hubbs makes special plea for the recognition of lower categories than subspecies, but without nomenclatural treatment. This might profitably be heeded by ornithologists and mammalogists who tend to encumber their taxonomy with very imperfectly differentiated subspecies. However, the ichthyologists' custom of reserving the term "race" for such minor categories should not be pressed upon other workers who for long have used "race" and "subspecies" as synonyms.

Intergradation, the *sine qua non* of the subspecific state to many vertebrate taxonomists, receives much attention. The more penetrating discussions (Hubbs, Dunn) of this

feature commendably acknowledge a wide variety of con-
ditions under this heading. I do not see that most types
of intergradation exclude the biologic factor of reproduc-
tive continuity, or the possibility of it; thus they are not
purely morphological phenomena as Mayr would seem to
imply.

Schism among the group of authors is sharpest on the
criteria for species. This is plain from Mayr's state-
ment: The species concept based ''on the criterion of mor-
phological distinctness or of a gap in morphological char-
acters is invalid. . . . The probability of reproductive
isolation is the primary criterion.'' With this stand
Blair would agree, but he would make his test of repro-
ductive isolation through laboratory experiments on fer-
tility. To the reviewer a physiologic isolation in nature
is quite as impressive as a failure of crossing of captive
animals. Also, I think there is as much chance of break-
ing down some fertility barriers as there is of breaking
certain psychological and other physiological isolations
operative in the wild. Should one type of reproductive
isolation be viewed as so much more significant than the
other? Blair, following the lead of the botanist Turesson,
distinguishes cenospecies in the mice of the genus *Pero-
myscus* in contrast to ''incipient species'' which are re-
productively isolated from one another in nature but
prove interfertile in forced matings.

Simpson would agree in principle with Mayr's view-
point on species, but in practice in dealing with fossil
material he is of course forced to follow morphological
criteria. Rather pedantically, he distinguishes the bio-
logic or genetic species from the morphological approxi-
mation to it that results from the prevailing parallel
between morphological differentiation and reproductive
isolation. And in turn, he distinguishes the taxonomist's
species based on available specimens, which species is
but an inference as to the nature of the true morphological
species. Simpson's paper, much the most extensive one
of the group, performs excellent service in clearly con-

trasting and relating vertical and horizontal species—a discussion well worth the careful attention of all paleontologists.   In defining a genetic species Simpson fails to preclude the subspecies, for he says the species is "a group of organisms so constituted and so situated in nature that a hereditary character of any one of these organisms may be (possibly, but not necessarily) transmitted to a descendant of any other."

Dunn and Hubbs emphasize morphological criteria, but it should be made clear that their position is in no way extreme in this direction.   Dunn states some of the objections to the admittedly logical and attractive criterion of mutual infertility: "it is usually impossible to apply it to the material with which" the taxonomist has to deal; "in many animals actual discontinuity of breeding may occur long before mutual infertility sets in . . . and cases are known in which two populations of what is demonstrably the same species [morphologically?] may manifest actual discontinuity of breeding and act toward each other as two species."

Hubbs perhaps wisely refuses to be bound by any one criterion and he concludes on this vein: "Even when all single tests for the species level break down, a form may be recognized as a species by reason of the usual validity of a series of criteria, just as some subspecies and species may be known by the usual though not invariable possession of each of a series of characters. . . . Neither conventionalized views nor subjective subterfuges—whether by the old-line systematist or by the modern speciationist —can transcend the facts, or create a simple 'correct' system of taxonomy or a simple theory of speciation out of a situation that is inherently complex."

Justice can scarcely be done in review to the many shades of meaning in the statements of the contributing authors.   Each has had a wealth of experience in his field and holds well considered opinions worthy of a hearing.   This symposium has served its purpose admirably.— ALDEN H. MILLER.

Systematics, Cytogenetics and Evolution in Crepis. By ERNEST B. BABCOCK. Bot. Rev., 8, 1942: 139–190.[1]

WITH tenacity of purpose Babcock and his associates have devoted twenty years of work to the study of the single plant genus *Crepis*. Although the final monograph awaits publication, many significant results have already been presented. I know of no large genus of animals that is now as well known, both taxonomically and cytogenetically, as *Crepis*. The recent work of Patterson, Sturtevant, Dobzhansky and Spencer promises that *Drosophila* may eventually surpass *Crepis* in this respect, but this goal is still in the far distance.

Crepis forms a compact genus, composed of 196 known species, which have descended from a common ancestral stock, probably somewhere in western Asia, and which have radiated into Africa, Europe, Asia and North America. With the help of morphological, ecological and cytological criteria it can be established rather reliably which species are primitive and which species belong to a single line of descent. This permits the study of evolutionary trends within sub-groups of the genus.

The special advantage of *Crepis* is that it has few chromosomes, which usually can be recognized individually. The emphasis of Babcock's work is therefore on cytogenetics. The primitive karyotype (chromosome pattern) of the genus consists in the haploid phase of 5 or 6 long, equi-armed chromosomes. Repeatedly and in independent lines this type tends to evolve toward one with fewer (3 or 4) and shorter chromosomes with uneven arms. Parallel, but not necessarily a result, is a change in morphology and life cycle from large and coarse perennials to small, delicate annuals. Gene and chromosomal mutations are common: "It appears that gross structural changes in the chromosomes have been of evolutionary importance mainly by producing intraspecific sterility barriers. . . . Differentiation within and between plant

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[1] Supplemented by: Genetic Evolutionary Processes in Crepis. By E. B. Babcock, G. L. Stebbins, Jr., and J. A. Jenkins. AM. NAT., 76, 1942: 337–363.

species depend mainly, if not wholly, upon gene muta-tions.'' Sterility is the only isolating factor that is dis-cussed in detail. A treatment of other mechanisms that may assist in the prevention of cross fertilization of re-lated sympatric species would have been of considerable interest. Or is sterility really the only isolating mecha-nism in *Crepis?*

Interspecific hybridization is rather frequent, but it is of secondary importance as a speciation process. Poly-ploids are few. The ordinary species concept, as derived from sexual diploids, breaks down in certain groups of hybridizing polyploids and associated agamic forms. Apomixis, frequently found in aggressive and wide-spread forms, leads to temporary advantages, but inevi-tably to eventual extinction.

The gene contents of not a single species of *Crepis* is anywhere nearly as well explored as that of *Drosophila melanogaster, Zea mays,* or *Gossypium.* As far as tax-onomy is concerned, the degree of perfection which intra-specific population analysis has reached in fishes, mam-mals and birds does not seem to be approached in *Crepis.* Filling this gap will require the statistical analysis of future mass collections. Still, Professor Babcock can point out with pride that he accomplished what he had set out to do: the classification of the species in a large and difficult genus and the establishment of the principal evo-lutionary processes that have led to this diversification of species. The *Crepis* studies represent a major contri-bution to the study of evolution.—ERNST MAYR.

The Structure and Origin of Species With a Discussion of In-traspecific Variability and Related Nomenclatural Problems. By W. H. CAMP AND C. L. GILLY. Brittonia, 4, 1943: 323–385.

PLANT systematists Camp and Gilly, of the New York Botanical Garden, openly engage the idea that the ''spe-cies problem'' is a problem of *the* 'species. Along with Huxley and certain other modern analysts of speciation they direct their attack on the different kinds of species.

They recognize a dozen categories, for which, ignoring previously proposed names, they erect the following terms, more or less strictly of Greek origin: homogeneon, phenon, parageneon, dysploidion, euploidion, alloploidion, micton, rheogameon, cleistogameon, heterogameon, and, for species in which apomixis is present, apogameon and agameon. The authors recognize but I believe underestimate the difficulties and disagreements that would arise in the practical application of this classification of species types. Perhaps because they are experienced taxonomists, they do not advocate revolutionary changes in nomenclatorial practice: seemingly they would apply the conventional binomial to all types of species. For routine systematics they favor only two intraspecific categories—the subspecies for those having geographical as well as morphological integrity and the forma for those, with some genetical continuity, which commingle with other types of the species. The less precise category of varietas they discredit, although they think it should probably not be abandoned in exploratory systematics. Strangely, they propose only one new intraspecific grouping, the phenogen, for units of a phenon which are intersterile but which can not be distinguished except by genetic analysis.

Camp and Gilly also propose a taxonomy for types of systematic research, stressing the newer approaches without discrediting routine or exploratory systematics. To more critical analyses unaccompanied by cytological or genetic analysis they restrict, I think unwisely, the term "general systematics." For taxonomic research involving cytology and genetics they provide a new designation, biosystematy. They regard this as *the* critical phase of systematics by which origins, relationships and taxonomic status are objectively demonstrable. I have found, on the contrary, that uncertainties in interpretation, particularly as to group ranking, are often accentuated by more extended and refined research. It seems to me that the prime value of experimental systematics lies in the revealing of speciational processes, and that

such processes are inherently so diverse and complex as to fit very poorly into fixed concepts of group ranking and nomenclature.

The references to recent treatises on the philosophy of plant systematics, and the critical remarks thereon, will prove of particular value to students of speciation.

The Biotic Provinces of North America. By LEE R. DICE.
    Ann Arbor: University of Michigan Press, 1943: i–viii, 1–78,
    Map 1. $1.75.

MERRIAM's "life zones" were long popular among students of geographical distribution, but are now commonly displaced by other areal units. In current studies the biotic divisions of North America are stressed. Dice's "biotic provinces" in particular override the life zones or belts. The general environment rather than temperature is thus stressed as the controlling factor in the natural, correlated distribution of plants and animals.

The boundaries of the biotic provinces from the transcontinental Eskimoan and Hudsonian in the north to the mosaic of units in the southwestern United States and in northern and central Mexico are admittedly generalized, but I think Dice understates the indefiniteness of many boundary lines when he writes "the facts of distribution would in some situations be almost as well satisfied if the boundary between two adjacent biotic provinces were moved ten, or, more rarely, as much as fifty, miles." The provinces as mapped are very broadly generalized, with little interdigitation and with intermixtures eliminated by definition. Perhaps this simplification of boundaries is the greatest fault of the new classification, as contrasted with the intricate pattern of life zones as mapped by Merriam's followers.

Without citation of evidence fresh-water communities are held to be included in the biotic provinces. In general, however, river systems are much more significant in the limitation of fresh-water faunas than are the boundaries of forests and other major vegetational types on

which the biotic provinces are largely based. In some places, however, the distribution of fishes transcends the limits of river systems to form major patterns somewhat corresponding with the recognized biotic provinces, particularly the Hudsonian, Canadian, and Austroriparian. The essential distinction of the southeastern and southwestern biotas of the United States is well confirmed by the distribution of fishes.

This latest contribution to regional biogeography, though scholarly in treatment, strong and modern in ecological concepts and thorough in historical considerations, shares with earlier general studies an emphasis on the distribution of large, conspicuous and "higher" organisms and maintains an approach that is essentially one of impression and opinion. How and where the boundaries between biotic provinces are to be drawn, which biotic areas are to be ranked as provinces rather than as districts or other subdivisions, how the provinces in turn are to be organized in larger units (which Dice makes no effort to do), and even whether the distributional patterns of various groups of animals and plants coincide sufficiently well to justify the recognition and mapping of general biotic provinces, are questions which I think can not be answered adequately until a very extensive accumulation of data has been subjected to thorough and critical statistical analyses.

NOTICES OF NEW BOOKS

Wildlife Refuges. By IRA N. GABRIELSON. New York: Macmillan Co., 1943: i–xiii, 1–257, figs. 1–17, pls. 1–32. $4.00. An accomplished naturalist as well as America's foremost wildlife administrator, Ira N. Gabrielson presents this detailed discussion of wildlife refuges as a sequel to his excellent book on "Wildlife Conservation" (1941). Under the initial leadership of Jay N. Darling and the succeeding administration of Dr. Gabrielson, and the immediate supervision of J. Clark Salyer, II, the national wildlife refuge system has become an effective force of preservation and restoration. As made clear in this excellent book, it is not only the game birds and mammals that are being

protected, but all forms of life. The refuge operations are gratifying examples of successfully applied ecology. As such they are contributing much to biology, particularly to the biology of populations, a subject of current interest to students of evolution.

Biochemistry and Morphogenesis. By Joseph Needham. Cambridge: at The University Press; New York: The Macmillan Co., 1942: i–xvi, 1–787, 328 illustrations. $12.50.—"Superb," "colossal" and other attributives ordinarily usurped by the motion-picture industry could rightly be applied to Needham's "Biochemistry and Morphogenesis." This book is a scholarly, masterful compendium and synthesis of the published information on the physiology of development, with particular reference to the chemical background. No one who is investigating the fundamental basis of morphogenesis can afford to be without this book. And when it is recalled that many leaders in biological thought have predicted that this is the field in which greatest progress is due in biology, the vast importance of Needham's work will be apparent.

Studies of Evaporation and Transpiration under Controlled Conditions. By Emmet Martin. Carnegie Inst. Wash. Publ. 550, 1943: i–iii, 1–48, figs. 1–17. $0.40 (paper bound, offset printing).—This contribution to plant physiology contains a discussion of ecological applications.

Seven Papers in Genetics and Physiological Genetics of Drosophila melanogaster. By Richard Blanc, Werner Braun, Eldon J. Gardner, Richard Goldschmidt, Claude A. Villee, Jr. Univ. Calif. Publ. Zool., 49, 1942: 1–183, pls. 1–11, 18 figs. $2.00. —This series of papers from Goldschmidt's productive laboratory further analyzes "the concept of gene action on the basis of relative rates, times, and thresholds of reaction." The first, by Richard Blanc, reporting "Observations on the Production of Wing Scalloping in Drosophila melanogaster," supports Goldschmidt's explanation rather than "Waddington's more complicated hypothesis." The same author with Werner Braun then treats "Phenocopies and X Radiation in Drosophila melanogaster," lining up the production of these mutation-simulating "morphoses" or "roentgenmorphoses" with the current rate theory

of development; statistical treatment is emphasized. Then Blanc, in collaboration with Claude A. Villee, Jr., deals with "The Effect of X Radiation upon Bristle Formation in Drosophila melanogaster," from much the same point of view. Werner Braun follows with "The Effect of Changes in the Time of Development on the Phenotype Mutants of Drosophila melanogaster." Eldon F. Gardner contributes "A Further Study of Genetic Modification of Dominance, Especially by Position Effects." The viewpoints of penetrance and expressivity are featured in Goldschmidt and Gardner's article, "A Further Contribution to the Analysis of Scalloped Wings in Drosophila melanogaster."

The final and longest contribution, by Claude A. Villee, Jr., is "A Study of Hereditary Homoeosis: the Mutant Tetraltera in Drosophila melanogaster." This investigation bears on problems of regeneration and homology as well as genetics. Certain phenotypes of the tetraltera mutant were found to duplicate almost exactly, in wing structure, the aberrant dipteran *Termitoxenia*.

Tertiary Prairie Grasses and Other Herbs from the High Plains. By MAXIM K. ELIAS. Special Papers, Geol. Soc. Am., 41, 1942; 1–176, pls. 1–17, fig. 1. $1.50.—Students of grasslands as well as paleobotanists and stratigraphers will find much of interest in this account of the origin or incursion and the subsequent evolution of the vegetation of the Great Plains. Students of evolution will also find material of significance, for the "comparative study of the fossil and living forms reveals evolutionary trends of the seeds of prairie grasses. The rather small and generalized Miocene ancestor gave rise to greatly diversified Pliocene and Recent species. The seeds of these include small and very large, very slender, and very stout forms, all of them variously adapted for protection against drought and for more efficient dispersal." Like many other paleontologists, Elias stresses the direct effect of the environment, and evolutionary trends which are not necessarily connected with minor fluctuating changes in the environments. A distinctly modern view is that interspecific hybridization has resulted "occasionally in stable mutations, subspecies or even new species (amphidiploids)." It is pointed out that such reticulate evolution complicates the phylogenetic picture. The family tree of the Stipeae is offered in a "spirit of symbolic interpretation." A brief discussion of "Taxonomy in Relation to Evolution" is included.

The Birds of Southern Veracruz, Mexico. By ALEXANDER WETMORE. Proc. U. S. Nat. Mus., 93, 1943: 215–340, pls. 26–28, fig. 11.—Although largely an annotated list of the birds, this paper contributes information on the general ecology of a little-known part of Middle America. The higher sections of two mountains in the Sierra de Tuxtla are placed in the Subtropical rather than the Humid or Arid division of the Tropical Zone, and "the Subtropical element here must be considered a remnant or fragment from the cooler climatic conditions of the Pleistocene." The relicts, however, are of tropical-mountain rather than northern derivation.

Biological Results of the Last Cruise of the Carnegie. By HERBERT W. GRAHAM and others. Carnegie Inst. Wash. Publ. 555, 1943: i–v, 1–92, 69 figs., 4 maps. $1.00 (paper), $1.50 (cloth).—Graham's paper on The Phytoplankton is probably the item of most general interest, certainly the most important oceanographical contribution, in the series of twelve reports.

SHORTER ARTICLES AND DISCUSSION
POPULATION STRUCTURE IN TOADS[1]

In the last decade it has become increasingly apparent that the development of diversity within species populations is intimately concerned with such factors as size of breeding populations, periodic fluctuation of population size, sex ratio, activity range and differential survival of progeny. The sum total of these factors, population structure, determines to a great extent the relative importance of the roles which mutation, natural selection and random fluctuation of gene frequency play in the genetic configuration of the species.

It is obviously desirable to secure adequate knowledge of population structure in a variety of living species representative of the animal and plant kingdoms. At present such comparative data are woefully lacking. It seems, therefore, worthwhile to place on record some pertinent data on population structure in toads. These data, referring chiefly to *Bufo americanus,* were collected by the writer in northeastern Oklahoma in the spring of 1941. The area investigated constitutes, roughly, a 75-mile east-west transect extending from Sand Springs, Oklahoma, to Kansas, Oklahoma. This transect includes portions of three of the biotic districts of Oklahoma as defined by W. F. Blair and Hubbell (1938) : Osage Savanna (western 1/5 of transect), Cherokee Prairie (middle 7/15 of transect), and Ozark (eastern 1/3 of transect). In the eastern one-third *B. americanus* occurs with *B. fowleri;* in the western two-thirds it occurs with *B. woodhousii.*

Observations on more than 50 ponds and pools from March 22 to May 26 showed 767 male *B. americanus* and 40 female *B. americanus.* None of these is a duplicate observation of the same individual, since all animals were marked by toe clipping when first captured. These figures are somewhat low for females, since they do not call and are less conspicuous in the ponds. In a number of instances, however, ponds were cleared of all evident females at night and then checked in the morning to

see how many egg masses had been deposited. Since females almost always oviposit the same night on which they come to the pond, the number of egg masses found in the morning is a rather accurate count of the female toads which were overlooked the night before plus such females as may have come to the pond after the survey. Results indicate that some females may be overlooked, the number depending upon the nature of the pool, but there is no indication that the sex ratio in the breeding pools is 1:1. Collections of non-breeding toads, however, show more nearly the expected 1:1 ratio. Evidently, then, females do not go to the breeding pools each year, and many of the calling males find no mates. Wright (1914) found that male *B. americanus* far outnumbered females in the breeding ponds around Ithaca, N. Y. In central Oklahoma Bragg (1940) found that male *B. woodhousii* outnumbered females in breeding congresses and concluded that females do not breed each year.

Because of the incompleteness of isolating mechanisms mixed breeding aggregations occur. Table 1 indicates all aggregations where both male and female toads were present (A refers to *B. americanus* and W to *B. woodhousii*). There is considerable opportunity for hybridization between *B. americanus* and *B. woodhousii*. No male *B. fowleri* was found in any of the *B. americanus* aggregations. The experience of the writer in northeastern Oklahoma indicates, however, that straggler males of *B. americanus* may occur in breeding populations of *B. fowleri*. No opportunity for studying this overlap was afforded during the present study. Because of the most severe flood on record or other causes there was no breeding of *B. fowleri* in the area under observation in 1941; observations throughout the summer showed a complete absence of tadpoles and young toads of this species.

The average size of breeding aggregations of *B. americanus* in the area under consideration is small. Reference to Table 1 shows that in only three out of 15 aggregations where both male and female *B. americanus* were found in the ponds were there more than 30 toads present. The average size of 15 aggregations was 19.3 toads (16.6 males and 2.6 females). In the area studied the breeding sites utilized by *B. americanus* are for the most part brook pools and temporary rain pools. ' With the exception of a limited number of flood plain sloughs the region contains few or no large natural pools which might be used for breeding. The largest breeding aggregation observed was in an artificial pond

north of Locust Grove, and it seems likely that the largest breeding populations are to be found in bodies of water impounded by man. At Ithaca, N. Y., the breeding congresses of *B. americanus* are evidently quite large, as Wright (1914) states that it is not a rare observation "to find a thousand toads in one small pond." The writer's observations (Blair, 1941) on *B. americanus* in Indiana indicate breeding populations intermediate in size between the small aggregations of northeastern Oklahoma and the very large ones of New York.

TABLE 1

COMPOSITION OF ALL BREEDING AGGREGATIONS (BOTH MALE AND FEMALE TOADS PRESENT) FOUND IN NORTHEASTERN OKLAHOMA IN 1941.

A refers to *B. americanus* and W to *B. woodhousii;* ♂A × ♀A indicates a clasping pair.

Locality	Type pool	Date	Toads present				
			♂A	♀A	♂A ×♀A	♂W	♀W
Locust Grove, Okla.	Shallow slough	March 22	10		1		
Locust Grove, Okla.	Large spring-fed pond	March 22	63	2	8		
Kansas, Okla.	Brook pool	March 23	9		1		
Kansas, Okla.	Brook pool	March 23	13	1	4		
Locust Grove, Okla.	Brook pool	March 31	13		1		
Sand Springs, Okla.	Pond	April 2	14	1	1		
Locust Grove, Okla.	Ditch puddle	April 15	2		1		
Scraper, Okla.	Ditch puddle	April 16	15		3		
Scraper, Okla.	Brook pool	April 16	12		2		
Kansas, Okla.	Brook pool	April 16	28	2	5		
Locust Grove, Okla.	Pool near creek	April 25	1			1	1
Kansas, Okla.	Brook pool	April 26	31		1		
Tulsa, Okla.	Brook pool	April 30	5				1
Tulsa, Okla.	Oil slush pond	April 30	2			4	1
Tulsa, Okla.	Pond	May 1	3	1		2	
Tulsa, Okla.	Rain pool	May 5	2			11	1
Tulsa, Okla.	Rain pool	May 7				8	1
Sand Springs, Okla.	Pond	May 20	3	1		1	
Locust Grove, Okla.	Large spring-fed pond	May 21	2		1		
Salina, Okla.	Backwater of creek	May 26	3	3			

Male toads which do not find mates may continue calling for some time. The following data for calling male *B. americanus* recaptured after being marked in the breeding pond give the number of days (numeral in parentheses) after being marked that the toad was retaken, and the number of toads (numeral outside of parentheses) retaken after a given number of days: (2)7, (3)2, (6)7, (7)3, (8)2, (9)8, (10)2, (11)2, (15)17, (17)1, (20)2, (22)1, (24)3, (26)1, (33)3, (34)2, (41)3, (42)1 and (51)1. Such records do not imply continuous nightly calling over the period indicated; unmated toads which have ceased calling may resume calling on the advent of rainfall.

The male chorus daily loses and gains members. For instance, 14 male *B. woodhousii* and one male *B. americanus* were calling

in a slough near Grand River on the night of April 25; of 13 male *B. woodhousii* and five male *B. americanus* calling in the same slough two nights later, seven *B. woodhousii* and one *B. americanus* were carry-overs from the first date. Of 18 male *B. ameri-*
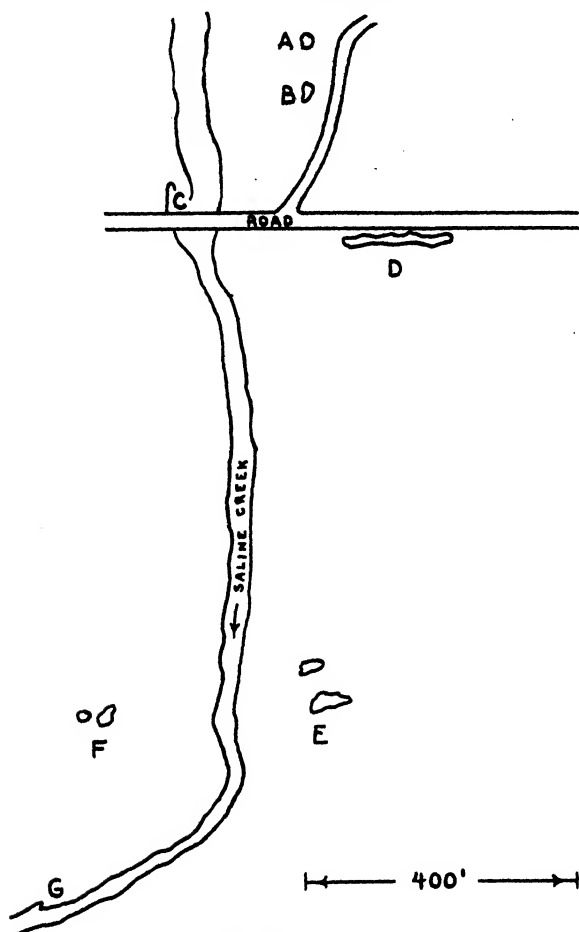
Fig. 1. Inter-pond migration of male *B. americanus;* see text for details.

canus calling in a ditch puddle southeast of Locust Grove on April 15, two were calling in a chorus of nine male *B. americanus* in the same puddle fifteen days earlier. The detailed history of a small area along Saline Creek, in the Ozark district, is given in the following compilation (see Fig. 1) :

March 31. Nine male *B. americanus* calling at A.

April 15. Of seven male *B. americanus* calling at A, two were marked at A March 31; of five male *B. americanus* calling at B, two were

marked at A March 31; of four male *B. americanus* calling at D, one was marked at A March 31.

April 17. Severe local flood covers area with 10-15 feet of water for several days.

May 3. Of two male *B. americanus* calling at A (pool about half filled with sand after flood), one was marked at A March 31.

May 11. Of 16 male *B. americanus* calling at E, one was marked at A March 31; of seven male *B. americanus* calling at F, two were marked at A March 31 and one at A April 15.

May 18. Of three male *B. americanus* calling at C, one was marked at F May 11.

May 21. Of five male *B. americanus* calling at E, one was marked at A March 31.

May 26. Of three calling male *B. americanus* and three female *B. americanus* at G, none was previously marked.

It is evident from this data that once arrived at a given pool male toads do not always remain until mated or until cessation of the reproductive urge leaves them unmated. The following tabulation lists male toads captured at breeding sites other than those where they were marked originally; the straight-line distance traveled and the time elapsed since marking are indicated:

Bufo americanus			*Bufo americanus* (cont.)	
	Distance (feet)	Time (days)	Distance (feet)	Time (days)
1.	45	15	13. 1,050	41
2.	45	15	14. 1,050	26
3.	350	15	15. 1,800	10
4.	390	33		
5.	400	42	*Bufo fowleri*	
6.	640	15	1. 250	9
7.	640	24	2. 250	12
8.	660	33	3. 260	3
9.	780	15	4. 300	3
10.	900	7	5. 660	7
11.	1,000	41	6. 810	8
12.	1,050	41	7. 1,100	8

In seven of these cases (*B. americanus* Nos. 7, 8, 10, 11, 12, 13 and 14) there was the possibility of passive movement by flood waters; the end achieved is the same, however. Two of the *B. americanus* (Nos. 6 and 9) must have gone by routes more than twice as long as indicated or else have crossed two ponds with a combined width of more than 400 feet. *Bufo fowleri* No. 7 had to swim the Illinois River, a stream 50–200 feet wide with a strong current at the narrower portions, to reach the point where it was recaptured. Such records indicate a considerable movement of

males from pond to pond during the breeding season. Piatt (1941) marked 120 mating pairs and 18 non-mating toads of *B. americanus* at four breeding sites of a 10-acre pond at Rensselaerville, New York; he recaptured only one toad, a female, at a breeding site different than that where marked.

SUMMARY

(1) Data on population structure in toads, chiefly *Bufo americanus*, were gathered in northeastern Oklahoma in the spring of 1941.

(2) In the area studied, *B. americanus* males greatly outnumbered females in the breeding ponds (6:1 considering all breeding aggregations where both male and female toads were present).

(3) Mixed aggregations of *B. americanus* and *B. woodhousii* occur.

(4) The average size of 15 breeding aggregations of *B. americanus* was found to be 19.3 toads (16.6 males and 2.6 females).

(5) The male chorus daily loses and gains members. Male toads which do not find mates may continue calling in the same pond or may migrate to another pond and call there.

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A HORMONE–PRODUCED TAXONOMIC CHARACTER IN PLATYPOECILUS MACULATUS DIAGNOSTIC OF WILD P. XIPHIDIUM[1]

THE adult males of *Platypoecilus xiphidium* Hubbs and Gordon of the Rio Soto la Marina in northeastern Mexico are distinguished from all other male members of their genus by a small, swordlike prolongation of several ventral rays of their caudal fins. The specific name of this platyfish was designed to emphasize this point. In a "key" to the four known species of *Platypoecilus,* the caudal sword may be used as a taxonomic character to separate *P. xiphidium* populations from those of *P. couchianus* (Girard) of the Rio San Juan System, *P. variatus* Meek of the Rio Panuco System and *P. maculatus* Günther of the Jamapa, Papaloapan, Coatzacoalcos and Usumacinta River systems. A brief survey of the speciation problems in the platyfish-swordtail group has been presented recently by Gordon (1943).

In the course of study of the effects of sex hormones upon developing sex characters and upon the courtship behavior of *P. maculatus,* a number of striking deviations from the normal were found by H. Cohen (1942). This discussion is concerned with the changes noted in the development of the caudal fin which were brought about by the use of the synthetic steroid, pregneninolone.[2]

For the purposes of these hormone studies it was held essential to use immature fish of known initial, genetic, sex. Since the external secondary sex characters in the platyfishes are not developed until approaching maturity (frequently a period of six to eight months) a genetic method for the determination of the sexes in the newly born was employed.

A black-spotted female *P. maculatus* of the constitution (W) + (Z)Sp, (New York Zoological Society Culture Number 8A-1), was mated with an unspotted male, (Z) + (Z) + (Culture Number 23-11). Ninety-six of their offspring were reared to maturity under normal conditions, that is, without the use of hor-

mones: 40 were spotted and males; 56 were unspotted and females. Deviations from these expected results are relatively infrequent, for according to Fraser and Gordon (1929) the cross-over value is about one per cent. (Parenthetically, it may be stated that endocrinologists who use fishes as their test animals may profit by the use of species in which sex may be determined early, either by genetic methods or by some early differentiating sexually dimorphic structures like those which Turner (1941) found in the anal fins of juvenile *Gambusia affinis*).

As part of these studies, fifteen unspotted, two-weeks-old, genetically determined, female *P. maculatus* of the same parents as indicated above were placed in three three-gallon aquaria. The water temperature was maintained at approximately 24° C. The fish were fed the usual types of food: dried shrimp, dried liver meal mixture and live tubifex worms. Every week they received, in addition, 5 mgm of pregneninolone crystals, some of which the fish appeared to swallow with the particles of their food. The hormone was given over a period of five months. The control fish received the identical food and were maintained under similar conditions.

The effects upon the pregneninolone-treated females were quite marked and were expressed in the changes seen in the gonads, muscles and various parts of the skeleton. The anal fin, unmodi-fied in the normal female, was transformed into a male-like gono-podium (confirming the work of Grobstein, 1940, 1941) and this organ was supported by a typical male-like gonopodial suspen-sorium and instrumented by a gonopodal muscle. The courtship behavior was typically male-like. In addition to all these effects of the androgenic hormone, a change in the structure of the ven-tral rays of the caudal fin was induced which is the main subject of this discussion.

The caudal fin of normal male and female *P. maculatus* is sym-metrical. The caudal fin of pregneninolone-treated females is asymmetrical: Some of the fin rays of the ventral sector are much shorter, while others are much longer than their counterparts in the dorsal sector. Specifically, rays number 7, 8 and 9 of the caudal fin, counting from the ventral to the dorsal region, are abnormally short, while ray number 6 is considerably longer than its dorsal counterpart. The shortening of some rays and the lengthening of another produces the appearance of the "sword" in the experimentally masculinized female *P. maculatus*. This

tail structure resembles the normal sword of the male *P. xiphidium.*

The platyfishes are closely allied to the swordtails of the genus *Xiphophorus* (see Hubbs and Gordon, 1943). The swordtails have "swords" of varying sizes: the male *X. hellerii* Heckel has the longest, about the length of its body; that of *X. montezumae* Jordan and Snyder is about half its body; while that of *X. pygmaeus* Hubbs and Gordon is tiny, shorter than that of *P xiphidium,* which is about as long as the width of its eye.

Female swordtails normally carry no swords, yet some, that have been known to produce offspring in their prime, develop short swords in their old age. Swords have been induced in young females by treatment with androgenic hormones, and in one case by estrones; for a detailed discussion of the problem the reader is referred to Witschi (1942). Furthermore, it has long been known that female swordtails carry hereditary factors for sword formation because they transmit these factors to their intergeneric hybrid sons when they are mated to male platyfish. It is possible to find among the many domesticated, aquarium-reared stocks of the platyfishes individuals which meet all the taxonomic criteria for *P. maculatus* except that some of the males may have a short sword, evidence of a promiscuous mating with the swordtail in the past. A genetic test for *X. hellerii* genes in aquarium stocks of *P. maculatus* has been briefly outlined by Gordon (1942).

The above leads us to postulate that it is likely that platyfishes, as well as swordtails, have genetic factors for sword formation. The genic complex for this structure varies with the species and with the sex of the species involved. These underlying complexes reflect the over-all genetic differences between the species. The normal manifestation of the hereditary factors for long sword, short sword or no sword at all are subject to changes under the influence of exogenous agents, specifically by treatment with unusual doses of sex hormones. Under these conditions, swords may be induced in female *X. hellerii* or in female *P. maculatus;* others of the group have not yet been tested.

In studies to discover the genetic nature of the differences between, and similarities in, the species of the platyfish-swordtail group some significant facts have been reported by Gordon (1943) concerning the distribution of a number of genes and their frequencies. In the light of this work and related details, the arti-

ficial transformation of a structure in one species of a xiphophorin fish to simulate a "key" character of another species is important only in that the experiment brings to light the basic genetic factors common to the group as a whole.

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